Taxonomy of Some Representatives of the Ophiostomataceae and Some Wood Staining Fungi Found in New Zealand

by

LEONARD JOSEPH HUTCHISON

A thesis submitted to the Faculty of Graduate Studies
in partial fulfilment of the requirements for the degree
Master of Science

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"You will find a fungus and determine its characteristics. You turn to the books and decide on its genus. Then you look for the species. And you look, and you look, and after a while you find it! In another genus!"

J.J. Davis fide Gilman (1953)

"Ingenious men will readily advance plausible arguments to support whatever theory they shall choose to maintain; but then the misfortune is, everyone's hypothesis is each as good as another's since they are all founded on conjecture."

Gilbert White fide Cooke (1962)

ABSTRACT

Fungi were isolated from stained wood collected from both native and introduced tree species growing on the North Island of New Zealand. The family Ophiostomataceae (9 species isolated) was the central focus of this taxonomic investigation, since species of the genus Ceratocystis which is assigned to that family are amongst the more important of the wood staining organisms. In addition, 3 species of wood-staining Coelomycetes, 12 species of wood-staining Hyphomycetes and one wood-staining Pyrenomycete were also isolated and studied.

Three new species of fungi, one of which represents a new genus, were isolated and described during this study: Ceratocystis novae-zelandiae sp. nov., Hyalopesotum pini sp. nov., and Mammariopsis gen.nov. (type species Mammariopsis variospora sp. nov.).

Each fungal isolate was subjected to a series of different cultural conditions to investigate the nature of ascocarp development in vitro. Near U.V. light induced mature perithecia in Coniochaeta velutina and immature ascocarps in Exophiala jeanselmei var. jeanselmei while extracts of Gliocladium roseum induced perithecial production in Ceratocystiopsis falcata. The latter fungus was studied in detail to determine the nature of this stimulation with results indicating that a simple protein in the G. roseum extract is most probably involved.

ACKNOWLEDGEMENTS

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I wish to thank the members of my committee, Dr. Alexander Olchowecki, Dr. David Punter, and Dr. John Mills for reading this thesis and providing much valuable advice.

The photographic plates of this thesis are a direct result of the very valuable guidance provided by Dr. Olchowecki with respect to techniques available for photomicrography and Dr. Punter is thanked for his preparation of the Latin diagnoses.

The quality of the typescript is due to the excellent typing skills of Ms. Barbara Lindsay.

And finally I wish to thank three of my colleagues in the Department of Botany who have shared with me their interest in fungi and whose friendship I gratefully acknowledge: Anthony Hopkin, Jeannie Gilbert, and Tawfik Muhsin.

TABLE OF CONTENTS

Page

APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

Because of its economic importance, wood staining by fungi has long been of interest in countries where forestry is important to the economy. Studies have, for the most part, been limited to the north temperate zone: Lagerberg et al. (1927), Melin and Nannfeldt (1934) and Mathiesen-Käärik (1960) in Sweden; Siemaszko (1939) in Poland; Münch (1907, 1908a, 1908b) in Germany; Goidànich (1936c) in Italy; Wright and Cain (1961), Griffin (1968), and Olchowecki and Reid (1974) in Canada; Hedgcock (1906), Rumbold (1931, 1936, 1941) and Davidson (1935, 1942, 1944, 1953, 1955, 1958, 1966, 1971, 1976, 1978) in the United States and Nisikado and Yamauti (1933, 1934, 1935) and Kitajima (1936) in Japan. However, this present study is based on a preliminary survey of wood staining fungi from New Zealand, a south temperate country where forestry is of a major economic importance.

The staining of wood by fungi is accomplished by the penetration into the wood of dark coloured mycelium or else the diffusion into the wood of dark coloured pigments produced by the fungus. Some stain fungi have the ability to do both.

Many fungi are responsible for staining wood of standing trees and cut timber, and these include species of Hyphomycetes, Coelomycetes and Pyrenomycetes. The most significant of these staining fungi belong in the genus Ceratocystis Ell. & Halst., which consists of over 100 species (Upadhyay, 1981)

collectively possessing very diverse anamorphic states (see Figs. 1a and 1b). Several such species are responsible for serious plant diseases including Dutch elm disease, caused by Ceratocystis ulmi (Buism.) C. Moreau, and oak wilt caused by Ceratocystis fagacearum (Bretz.) Hunt in which infections are induced by both conidia and ascospores. However, many species are saprophytes attacking the medullary rays and parenchyma cells of dead or dying trees; in the process, a characteristic bluish to almost black staining of the wood develops.

Fruiting structures of those Ceratocystis spp. which attack woody hosts are often found in bark-beetle galleries on the host, and here both anamorphic and teleomorphic states develop.

The perithecia characteristically have a globose venter, frequently surmounted with a long neck which has a neck canal creating a continuous channel between the ostiole and the venter cavity. However, some species possess very short necks, while a few have no necks at all. The deliquescent asci develop throughout the centrum tissue and passively release the ascospores into the venter; the ascospores may be of various shapes (see Fig.2). Due to internal pressure in the centrum, the ascospores are extruded through the ostiole in a sticky matrix. Because of the location where the fruiting bodies are produced and the sticky nature of the ascospores, these fungi are well adapted to have bark-beetles serve as the main vectors of spore dispersal (Verrall, 1941). The beetle comes into contact with the spore mass at the

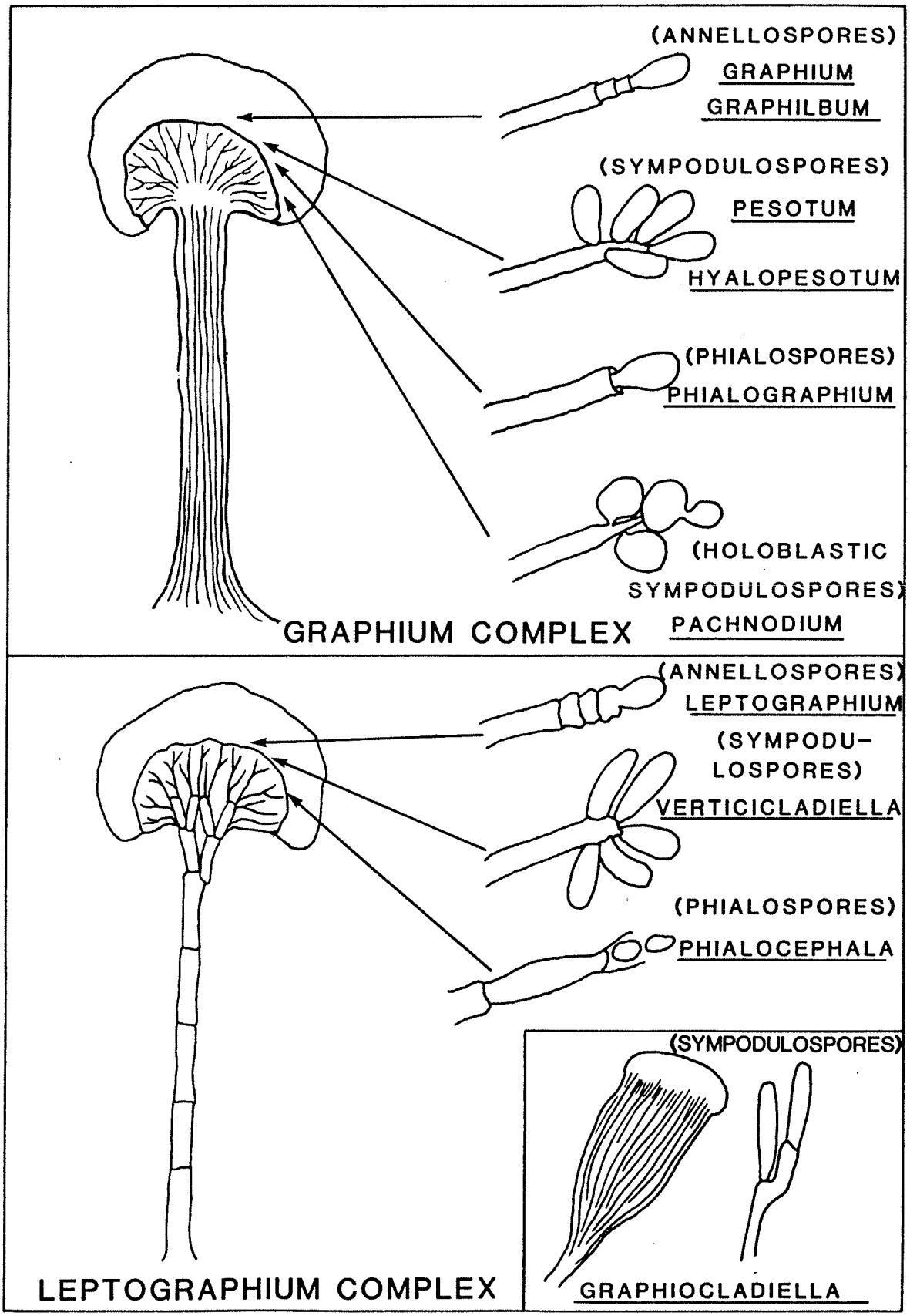


FIGURE 1a ANAMORPHIC STATES OF CERATOCYSTIS AND CERATOCYSTIOPSIS

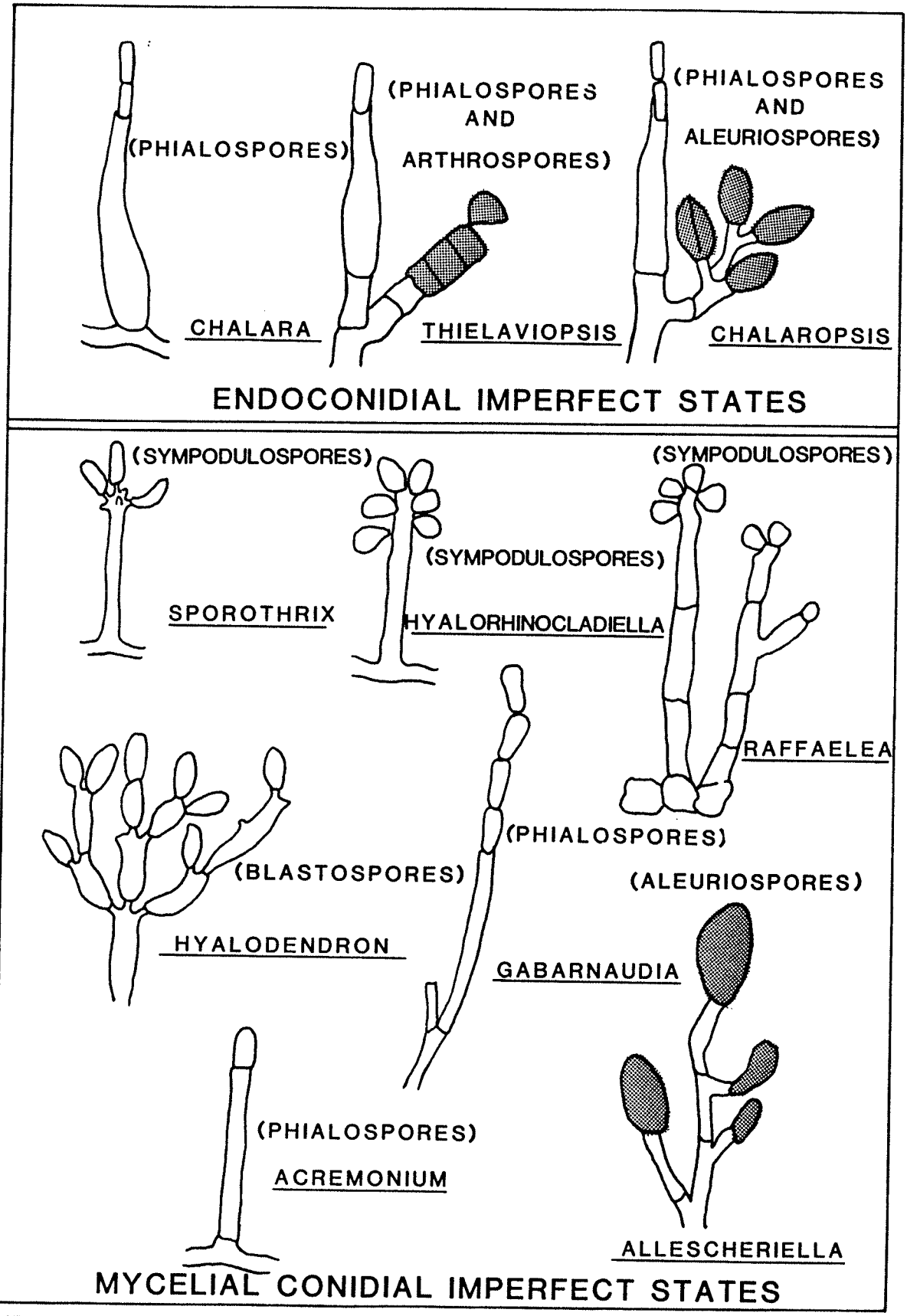


FIGURE 1b. ANAMORPHIC STATES OF CERATOCYSTIS AND CERATOCYSTIOPSIS

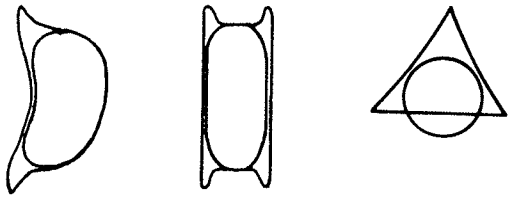
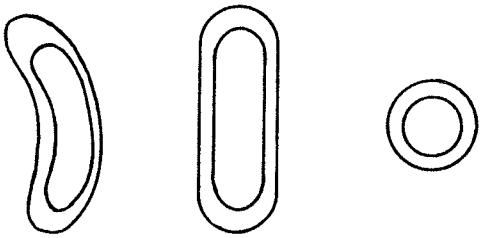
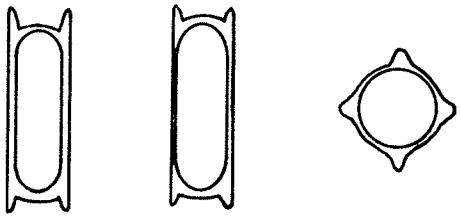
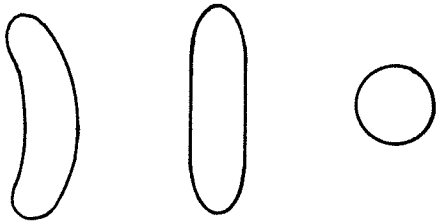
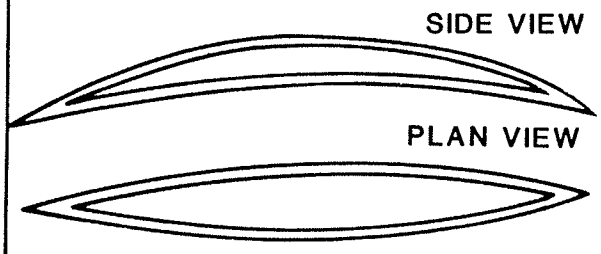
MORPHOLOGY OF ASCOSPORES SIDE VIEW PLAN VIEW END VIEW	SUBDIVISION UPADHYAY (1981)	 OLCHOWECKI AND REID (1974)
	SECTION: CERATOCYSTIS	GROUP: FIMBRIATA
	SECTION: ENDOCONIDIO–PHORA	NO EQUIVALENT (FOUND IN OTHER GROUPS)
	SECTION: IPS	GROUP: IPS
	SECTION: OPHIOSTOMA	GROUP: PILIFERA
	GENUS: CERATO–CYSTIOPSIS	GROUP: MINUTA

FIGURE 2. SUBDIVISION OF THE GENUS CERATOCYSTIS ON THE BASIS OF ASCOSPORE MORPHOLOGY

tip of the neck of the fructification and the spores then become adherent to the passing beetle; the relationship between the fungus and the bark-beetle is considered by some workers to be symbiotic (Whitney, 1982).

The anamorphic states are also found in bark-beetle galleries either prior to, or in association with, the perithecia and, depending upon the species, are extremely variable in their nature. However, since many anamorphs also produce spores in a sticky matrix, the asexual states are also often adapted for beetle dispersal.

LITERATURE REVIEW

(A) Taxonomic History of the Genus *Ceratocystis*[1]

The nomenclatural history of the genus is very complicated and confusing with Figure 3 showing the many changes that took place relative to the type species alone, in a span of 90 years.

Although Ellis and Halsted proposed the generic name *Ceratocystis* in 1890 (Halsted, 1890), the type species *Ceratocystis fimbriata* Ell. & Halst. was not described until the following year (Halsted and Fairchild, 1891). Because of the deliquescent nature of the asci, Halsted and Fairchild misinterpreted ascocarps as pycnidia and ascospores as conidia. This caused Saccardo (1892) to place *C. fimbriata* into the genus *Sphaeronaema* Fr. as *S. fimbriatum* (Ell. & Halst.) Sacc. Elliott (1923) examined the purported pycnidia of *S. fimbriatum* and determined that the conidia were really ascospores liberated within the perithecia by early disintegration of the asci and he then transferred the species to the genus *Ceratostomella* Sacc. as *Ceratostomella fimbriata* (Ell. & Halst.) Elliott. Nannfeldt (Melin and Nannfeldt, 1934) decided this species belonged to the genus *Ophiostoma* Syd. & Syd. and so placed it as *Ophiostoma fimbriatum* (Ell. & Halst.) Nannf. However, because of its endoconidial (*Chalara*) imperfect state, Davidson (1935) soon afterwards created a new combination *Endoconidiophora fimbriata* (Ell. & Halst.) Davids. Finally it was

[1]The taxonomic history of the other groups of fungi are discussed separately in the discussion for each species.

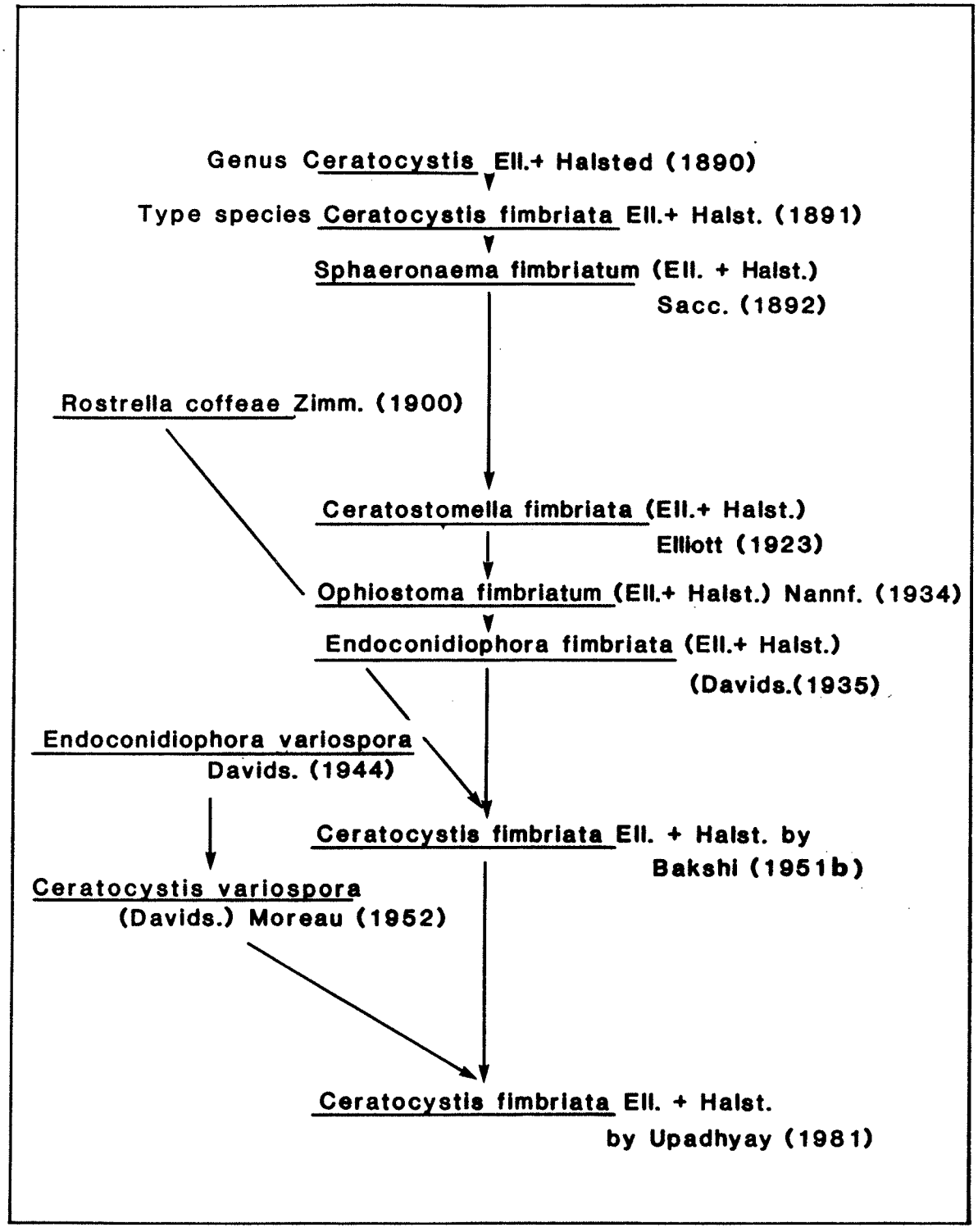


Figure 3. Chart showing sequence of changes and corresponding synonyms for the type species of Ceratocystis.

transferred back to the genus Ceratocystis by Bakshi (1951b). In addition, two other species, Rostrella coffeae Zimm. (1900) and Ceratocystis variospora (Davids.) C. Moreau (1952) were reduced to synonymy with C. fimbriata by Bakshi (1951b) and Upadhyay (1981), respectively.

The confusing nomenclatural history of the genus Ceratocystis is outlined in Figure 4.

Ceratostomella was established by Saccardo (1878) based on the type species Ceratostomella lejocarpa Sacc. In 1900 Zimmermann erected Rostrella gen. nov., type species Rostrella coffeae Zimm., and later this would be treated as a synonym of Ceratocystis. Next Endoconidiophora Münch (1907) was erected to segregate out those species of Ceratostomella possessing an endoconidial asexual state (Chalara), while Höhnel (1918) proposed the generic name Linostoma to segregate species of Ceratostomella with deliquescing asci and ostiolar hyphae; thus Linostoma Höhn. differed from the type species of Ceratostomella, C. lejocarpa, since the latter had persistent asci and lacked ostiolar hyphae. However, Linostoma Höhn. was a later homonym of Linostoma Wallich (1831), a flowering plant genus, so Sydow and Sydow (1919) proposed the name Ophiostoma to replace Linostoma Höhn.

In 1934 Nannfeldt (in Melin and Nannfeldt) subdivided the genus Ophiostoma into two sections: (a) Brevirostrata for species which possessed short-necked perithecia, and (b) Longirostrata for those species which possessed long-necked perithecia; Longirostrata

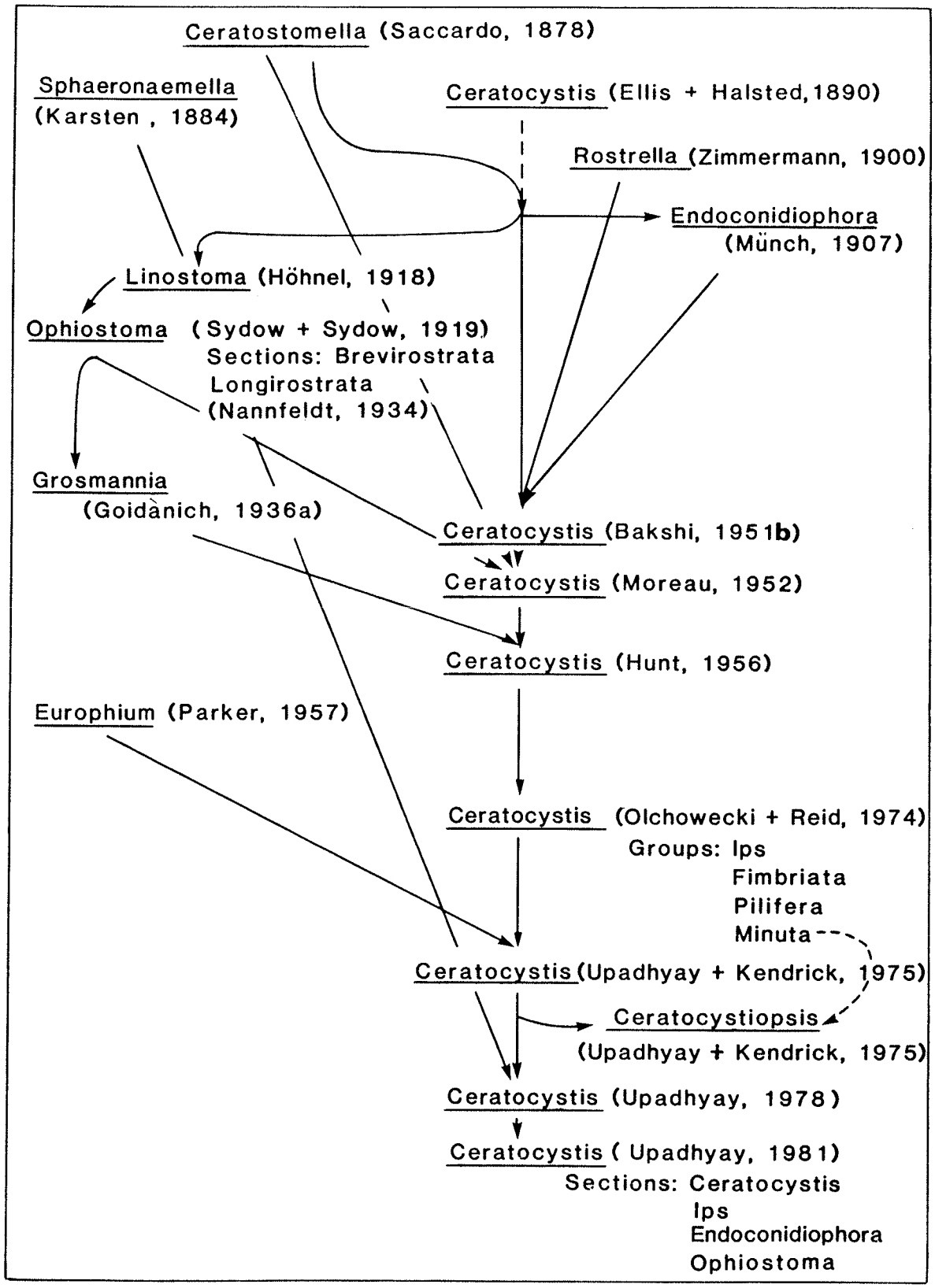


Figure 4. Flow chart history of nomenclatural concepts of the present day genus Ceratocystis. For explanation refer to text.

was further subdivided depending on whether the asexual states produced endo- or exoconidia. However, Goidànich (1936a) erected the genus Grosmannia to segregate out those species previously placed in the genus Ophiostoma but which possessed Leptographium-like conidial states.

Bakshi (1951b) considered that Rostrella and Endoconidiophora were synonymous with Ceratocystis. Further, although Grosmannia was originally segregated from Ophiostoma because its species possessed Leptographium-like imperfect states, and Ophiostoma spp. did not, Bakshi felt Grosmannia was not an acceptable genus because many species placed in the genus Ophiostoma have Leptographium-like imperfect states as well as other anamorphic states. He felt the genus Ceratostomella was a distinct genus because it possessed persistent asci.

Moreau (1952) reviewed the taxonomic history of the genus and transferred many species from the genera Endoconidiophora, Ceratostomella and Ophiostoma into the genus Ceratocystis based on the similarity of their imperfect states.

Hunt (1956) was the first to attempt a monograph of Ceratocystis and he completed the transfer of species from Endoconidiophora, Ophiostoma, Grosmannia and Ceratostomella using deliquescing asci, irregularly arranged within the perithecia as the main taxonomic criteria delimiting Ceratocystis. He divided Ceratocystis into three sections, each characterized by one of the following conidial state forms: (1) species with endoconidial

imperfect states; (2) species with Graphium-like or Leptographium-like imperfect states; and (3) species producing only mycelial conidia.

In 1957, Parker erected the genus Europhium for those Ceratocystis-like species which do not develop perithecial necks. Mathiesen-Käärik (1960), on the other hand, who undertook a detailed taxonomic study of Swedish isolates of Ceratocystis, and provided much useful information on the taxonomy and biology of these species, basically used the taxonomic characters delimited by Hunt. However, Wright and Cain (1961), who described 4 new species, used the morphology of the ascospores and whether they possessed a gelatinous sheath as the main taxonomic characters; the shape and size of the perithecia were considered to be of secondary importance. Griffin (1968), who described 11 new species, also used ascospore morphology for the separation of species, and Olchowecki and Reid (1974) described 25 new species, using Griffin's ascospore types to create 4 groups within the genus: (1) Minuta; (2) Ips; (3) Fimbriata; and (4) Pilifera. Into one of these groups each species was placed depending on its specific ascospore morphology; as well, they also stressed the method of conidium formation in the determination of species relationships.

Upadhyay and Kendrick (1975) erected the genus Ceratocystiopsis, this was equivalent to the Minuta ascospore group of Olchowecki and Reid, and reduced the genus Europhium to synonymy with Ceratocystis. Subsequently, in 1978, Upadhyay proposed that

the genus Sphaeronaemella Karst. should be reduced to synonymy with Ceratocystis. Sphaeronaemella had been erected by Karsten (1884) for fungi which had light-coloured, long-necked perithecia and deliquescing asci; however Upadhayay felt that perithecial pigmentation was not an important generic criterion because many species of the genus Ceratocystis have light-coloured perithecia.

In his monograph of the genus Ceratocystis, Upadhyay (1981) retained Ceratocystiopsis as a separate genus, formally transferred the species of the genus Sphaeronaemella to Ceratocystis, and, in a manner similar to that of Olchowecki and Reid (1974), divided Ceratocystis into 4 sections based on ascospore morphology. He also placed special emphasis on conidiogenesis and pleomorphism in the conidial state characters and reduced many species to synonymy; thus he had 75 species in the genus Ceratocystis and 15 species in the genus Ceratocystiopsis.

Historically, many European mycologists have not accepted the reduction of Ophiostoma to synonymy with Ceratocystis. This is especially true of mycologists in the Netherlands and Germany who feel that the name Ceratocystis should only be applied to those species with an endoconidial imperfect state, with the name Ophiostoma being retained for the majority of the remaining species (de Hoog, 1974; von Arx, 1974). de Hoog and Scheffer (1984) put forward a number of points which they believed were reason enough to keep Ophiostoma separate from Ceratocystis. Besides having

conidial anamorphs other than Chalara, Ophiostoma species have rhamnose and cellulose in their cell walls, and are resistant to cycloheximide whereas Ceratocystis spp. are unable to grow on media with cycloheximide and lack rhamnose and cellulose in their cell walls.

(B) The Anamorphic States of Ceratocystis Species

An appreciation of the diverse nature of the imperfect states of species of the genera Ceratocystis and Ceratocystiopsis, has been slow to develop. The most recent total (Upadhyay, 1981) indicates there are 16 discrete genera of the Hyphomycetes into which the various anamorphic states can be placed (Figs. 1a and 1b) and these anamorphic entities produce a variety of different types of conidia i.e. sympodulospores, annellospores, blastospores, phialospores, and, in some cases, they may produce two distinct developmental types of conidia on the same conidiophore. It has also been documented that members of the genus Ceratocystis may possess more than one anamorphic state. However, traditionally these imperfect states have been placed into one of three broad groups: (1) those with a Graphium- or a Leptographium-like imperfect state; (2) those with endoconidial imperfect states; and (3) those with mycelial conidial states.

The mononematous Leptographium complex consists of 3 types, generally similar in gross appearance, and differing only in the manner in which the conidia are produced.

Long after it was initially described, Leptographium lundbergii Lagerb. & Melin, the type species of Leptographium Lagerb. & Melin (in Lagerberg et al., 1927) was found to produce its conidia from annellides. Because of this, those fungi with mononematous conidiophores previously referred to the Leptographium complex but producing sympodulospores, were assigned to the genus Verticicladiella Hughes (1953), type species V. abietina (Peck) Hughes; Verticicladiella was treated in detail by Kendrick (1962) who recognized 7 species. In 1961, Kendrick erected Phialocephala Kendr., type species P. dimorphosa Kendr., for species of Leptographium-like fungi which actually produced their conidia from phialides.

The Graphium complex, consisting of fungi whose conidiophores are aggregated into synnemata, comprises six superficially similar anamorph genera; however variations in the way conidia are produced and whether synnemata are pigmented have long been recognized.

Species of Graphium Corda (1837), type species G. penicillioides Corda, produce their conidia on annellides, as do members of Graphilbum Upadh. & Kendr. (1975), type species G. sparsum Upadh. & Kendr., which is considered to be the hyaline analogue of Graphium. Species of Pesotum Crane & Schoknecht (1973), type species P. ulmi (Schwarz) Crane & Schoknecht, produce sympodulospores and this was the basis of its separation from Graphium. Hyalopesotum Upadh. & Kendr. (1975), type species H. introcitrina Upadh. & Kendr., was erected as the hyaline analogue of Pesotum.

Those members of the Graphium complex which produce their conidia from phialides are placed in the genus Phialographium Upadh. & Kendr. (1974), type species P. sagmatosporae Upadh. & Kendr., while the genus Pachnodium Upadh. & Kendr. (1975), type species P. canum Upadh. and Kendr., comprises species which produce holoblastic sympodulospores.

An anamorphic form which cannot be classified as a member of either the Graphium or Leptographium complex is the monotypic genus Graphiocladiella Upadh. (1981), type species G. clavigerum Upadh.; this produces sympodulospores on mononematous and/or synnematous conidiophores.

Those genera producing endoconidia usually possess simple conidiophores bearing phialides from which the endogenous spores are extruded in basipetal succession.

Thielaviopsis Went (1893), type species T. ethaceticus Went, and Chalaropsis Peyronel (1916), type species C. thielavioides Peyronel, are distinguished from Chalara (Corda) Rabenh. (1844), type species C. fusidioides (Corda) Rabenh., because species of Chalaropsis also produce aleuriospores, while Thielaviopsis spp. produce arthrospores as their second spore state. El-Ani (1958), however, felt that Chalaropsis should be reduced to synonymy with Chalara because it only required a single gene mutation for Chalaropsis to lose its ability to produce aleuriospores and Barron (1968) believed that both Thielaviopsis and Chalaropsis represented synonyms of Chalara; Nag Raj and Kendrick (1975)

formally proposed this synonymy.

The imperfect states of Ceratocystis and Ceratocystiopsis found in the mycelial conidial group include seven anamorph genera.

Species of Sporothrix Hektoen & Perkins (1900), type species S. schenkii Hektoen & Perkins, a fungal pathogen of humans, possess conidiophores which produce sympodulospores upon denticles. Species of the genus Hyalorhinocladiella Upadh. & Kendr. (1975), type species H. minuta-bicolor Upadh. & Kendr., also produce sympodulospores but, rather on denticles, they are produced directly from the conidiophores.

Acremonium Link ex Fr. (1821), type species A. alternatum Link ex S.F. Gray, comprises species producing phialospores in an enteroblastic manner on erect, slender hyaline conidiogenous cells. Acremonium was formerly referred to as Cephalosporium Corda (1839) but in 1971 Gams took up the older generic name Acremonium.

Representatives of Allescheriella Hennings (1897), type species A. crocea (Mont.) Hughes, possesses terminal holoblastic conidia while species of Hyalodendron Diddens (1934), type species H. lignicola Diddens, produce blastospores in acropetalous chains. A Hyalodendron sp. was first reported as a conidial state of a Ceratocystis sp. by Goidanich (1935).

Gabarnaudia Samson & Gams (Samson, 1974), type species G. betae (Delacroix) Samson & Gams, has four species producing enteroblastic conidia from phialides. Gabarnaudia species are

the anamorphic states of Sphaeronaemella spp. and whether they should be considered anamorphs of Ceratocystis depends on whether one agrees with Upadhyay's reduction of Sphaeronaemella to synonymy with Ceratocystis.

Weijman and de Hoog (1975) also consider the genus Raffaelea von Arx & Hennebert (1965), type species R. ambrosiae von Arx & Hennebert to include the imperfect states of certain Ceratocystis spp.; Upadhyay (1981) believes this is incorrect.

(C) Perithecial Formation In Vitro

Although most ascomycetous fungi grow quite well in culture, usually producing anamorphic spores, perithecial development in vitro is variable. Further, even those which produce ascocarps in culture initially often lose that ability after repeated transferring (Müller, 1979; Upadhyay, 1981).

Genetic control mechanisms may also govern ascocarp production. If one has isolated only a single mating type of a heterothallic species, the teleomorph state will not develop. This problem was first demonstrated in a Ceratocystis sp. by Dade (1928) who studied Ceratocystis paradoxa (Dade) C. Moreau, and soon afterwards other species of the genus were reported to be heterothallic, e.g. C. ulmi by Buisman (1932), Ceratocystis pluriannulata (Hedgc.) C. Moreau by Gregor (1932) and Ceratocystis multiannulata (Hedgc. & Davids.) Hunt by Davidson (1940).

In addition to compatability mechanisms, it has long been

known that a variety of environmental factors play an important role in governing the ability of a fungus to fruit in culture, e.g. temperature, pH, light, and nutrition (Müller, 1979).

The minimum, optimum and maximum levels of temperature and pH differ for different species of Ceratocystis, but Upadhyay (1981) suggests that generally a slightly acidic pH of 6.0 and a temperature range of 22-25^{o}C are best for the formation of perithecia. However, Verrall (1939) and Lea and Brasier (1983) have shown that under natural conditions in milder climates, some Ceratocystis spp. tend to produce their perithecia during the cooler winter months and produce only vegetative growth during the hotter summer period.

With respect to light stimulation of perithecial formation, Müller (1979) reports that near UV and long wavelength UV have the greatest demonstrated influence on fungi as a whole, although the effects of light may be modified by other factors such as temperature or nutrition.

Nutrition plays a significant role in the stimulation of perithecia in fungi, and this is true for the various species of Ceratocystis.

Käärik (1960) found that nutrient concentration is of great importance, e.g. a high carbon/nitrogen ratio would induce perithecia in various species of Ceratocystis but the required C/N ratio is not the same for all species.

A number of Ceratocystis species only produce perithecia in vitro if grown on autoclaved pieces of wood. This was demonstrated by Olchowecki and Reid (1974) with such species as Ceratocystis cainii Olchow. & Reid and Ceratocystis torticiliata Olchow. & Reid.

Early workers such as Heald and Pool (1908), McCormick (1925), Wilson (1927), and Asthana and Hawker (1936) found that fungi would produce ascocarps if they were grown in mixed cultures or if extracts of other fungi were added to the growing medium. This soon led to the realization that some fungi are able to synthesize their vitamin requirements from the media upon which they are growing, while others can not. Therefore, in mixed cultures, those fungi which could synthesize their vitamin requirements often would produce an excess which was made available exogenously to those fungi which could not synthesize a particular vitamin(s). This enabled vitamin deficient fungi to grow and fruit. Since these early reports, many workers have studied the vitamin requirements of various species of fungi and it was about 40 years ago that the vitamin requirements for perithecial production in various Ceratocystis spp. was first investigated.

Robbins and Ma (1942a, 1942b, and 1943) studied 23 species of Ceratocystis and found that the required vitamins were thiamine, pyridoxine and biotin. Some species required only one vitamin, some required two and some required all three before perithecial production was stimulated. Mathiesen (1950a) studied the vitamin requirements of Ceratocystis minor (Hedgc.) Hunt and later, invest-

igated a further 16 Ceratocystis spp. (Kääarik, 1960); she found the same trends as Robbins and Ma. She also noted that different strains of the same species were not identical in their vitamin requirements. Other workers (Fries, 1943; Barnett and Lilly, 1947; Campbell, 1958; Wikberg, 1959) have demonstrated that vitamins are required for perithecial formation in various other Ceratocystis species. Results of the above workers are summarized in Table 1.

Recently it has been shown that the addition of fatty acids to the media can promote perithecial production in species of Ceratocystis. Dalpé and Neumann (1976) and Marshall et al. (1982) felt perithecial production was stimulated the most with the addition of linoleic acid.

TABLE 1. VITAMIN REQUIREMENTS OF THE VARIOUS SPECIES OF *CERATOCYSTIS*

CERATOCYSTIS SPECIES NAME (UPADHYAY, 1981)	NAME ORIGINALLY TESTED UNDER	VITAMIN REQUIREMENT*			AUTHORS
		THIAMINE	PYRIDOXINE	BIOTIN	
CERATOCYSTIS ADIPOSA	ENDOCONIDIOPHORA ADIPOSA	—	—	—	ROBBINS AND MA 1942 b)
CERATOCYSTIS BRUNNEO - CILIATA	OPHIOSTOMA BRUNNEO - CILIATUM	—	—	PARTIAL	KAARIK (1960)
CERATOCYSTIS CANA	OPHIOSTOMA CANUM	PARTIAL	+	+	KAARIK (1960)
CERATOCYSTIS COERULESCENS	ENDOCONIDIOPHORA COERULESCENS	PARTIAL	—	—	ROBBINS AND MA (1942 b)
	OPHIOSTOMA COERULESCENS	+	—	—	KAARIK (1960)
CERATOCYSTIS FIMBRIATA	CERATOSTOMELLA FIMBRIATA	+	PARTIAL	PARTIAL	ROBBINS AND MA (1942 a)
	CERATOSTOMELLA FIMBRIATA	+	—	—	BARNETT AND LILLY (1947)
	CERATOCYSTIS VARIOSPORA	+	—	—	CAMPBELL (1958)
CERATOCYSTIS IPS	CERATOSTOMELLA IPS	+	+	+	ROBBINS AND MA (1942 a)
	CERATOSTOMELLA MONTIUM	+	+	+	ROBBINS AND MA (1942 a)
	CERATOCYSTIS IPS	+	+	+	KAARIK (1960)
CERATOCYSTIS LEPTOGRAPHIOIDES	CERATOSTOMELLA LEPTOGRAPHIOIDES	—	—	PARTIAL	ROBBINS AND MA (1943)
CERATOCYSTIS MICROSPORA	CERATOSTOMELLA MICROSPORA	+	+	+	ROBBINS AND MA (1942 b)
CERATOCYSTIS MINOR	CERATOSTOMELLA PINI	+	—	+	ROBBINS AND MA (1942 a)
	CERATOSTOMELLA PSEUDOTSUGE	PARTIAL	PARTIAL	—	ROBBINS AND MA (1942 a)
	OPHIOSTOMA PINI	+	—	+	FRIES (1943)
	OPHIOSTOMA PINI	+	—	+	MATHIESEN (1950a)
	OPHIOSTOMA PINI	+	—	+	KAARIK (1960)
CERATOCYSTIS MULTIANNULATA	CERATOSTOMELLA MULTIANNULATA	PARTIAL	+	—	ROBBINS AND MA (1942 b)
	OPHIOSTOMA MULTIANNULATA	+	+	—	FRIES (1943)
	OPHIOSTOMA MULTIANNULATA	—	+	—	WIKBERG (1959)
CERATOCYSTIS OLIVACEA	OPHIOSTOMA OLIVACEUM	+	—	+	KAARIK (1960)
CERATOCYSTIS PARADOXA	ENDOCONIDIOPHORA PARADOXA	+	—	—	ROBBINS AND MA (1942 b)
	CERATOSTOMELLA PARADOXA	+	—	—	ROBBINS AND MA (1943)
CERATOCYSTIS PENICILLATA	CERATOSTOMELLA PENICILLATA	+	—	+	ROBBINS AND MA (1942 b)
	OPHIOSTOMA PENICILLATUM	+	PARTIAL	+	KAARIK (1960)
CERATOCYSTIS PICEAE	OPHIOSTOMA CATONIANUM	PARTIAL	+	—	ROBBINS AND MA (1942 b)
	OPHIOSTOMA PICEAE	+	—	—	FRIES (1943)
	OPHIOSTOMA QUERCUS	+	—	—	FRIES (1943)
	OPHIOSTOMA FAGI	—	+	—	FRIES (1943)
	OPHIOSTOMA PICEAE	—	+	PARTIAL	KAARIK (1960)
	OPHIOSTOMA FLOCCOSUM	—	+	PARTIAL	KAARIK (1960)
CERATOCYSTIS PICEAPERDA	CERATOSTOMELLA PICEAPERDA	—	PARTIAL	PARTIAL	ROBBINS AND MA (1942 a)
CERATOCYSTIS PILIFERA	CERATOSTOMELLA PILIFERA	PARTIAL	PARTIAL	+	ROBBINS AND MA (1942 b)
	OPHIOSTOMA PILIFERUM	—	+	—	FRIES (1943)
	OPHIOSTOMA COERULEUM	+	—	—	FRIES (1943)
	OPHIOSTOMA COERULEUM	—	+	PARTIAL	KAARIK (1960)
CERATOCYSTIS PLURIANNULATA	CERATOSTOMELLA PLURIANNULATA	PARTIAL	+	—	ROBBINS AND MA (1942 b)
CERATOCYSTIS RADICICOLA	CERATOSTOMELLA RADICICOLA	+	—	+	ROBBINS AND MA (1943)
CERATOCYSTIS ROSTROCYLINDRICA	CERATOSTOMELLA ROSTROCYLINDRICA	DEFICIENCY UNKNOWN			ROBBINS AND MA (1942 b)
CERATOCYSTIS SAGMATOSPORA	CERATOSTOMELLA OBSCURA	+	—	+	ROBBINS AND MA (1942 b)
CERATOCYSTIS STENOCERAS	CERATOSTOMELLA STENOCERAS	+	—	—	ROBBINS AND MA (1942 b)
	OPHIOSTOMA STENOCERAS	+	—	—	FRIES (1943)
	OPHIOSTOMA ALBIDUM	+	+	PARTIAL	KAARIK (1960)
	OPHIOSTOMA STENOCERAS	PARTIAL	—	—	KAARIK (1960)
CERATOCYSTIS TETROPII	OPHIOSTOMA TETROPII	+	+	PARTIAL	KAARIK (1960)
CERATOCYSTIS ULMI	CERATOSTOMELLA ULMI	—	+	—	ROBBINS ANOMA (1942 a)
	OPHIOSTOMA ULMI	—	+	—	FRIES (1943)
	OPHIOSTOMA ULMI	—	+	—	KAARIK (1960)
DOUBTFUL SPECIES	OPHIOSTOMA CLAVATUM	—	PARTIAL	+	KAARIK (1960)
	GROSMANNIA SERPENS	—	—	+	ROBBINS AND MA (1942 b)
	CERATOCYSTIS GALEIFORMIS	PARTIAL	PARTIAL	+	KAARIK (1960)

NOTE: + = COMPLETE REQUIREMENT, — = NO REQUIREMENT.

(A) TAXONOMIC INVESTIGATIONS

METHODS AND MATERIALS

Wood samples were collected during the months of May and June, 1982 (late fall, early winter in the southern hemisphere) from various areas of the North Island (Figure 5) from both native and exotic forests. These samples were obtained from standing or recently cut trees with still adhering bark, and always from areas showing evidence of bark-beetle activity. These samples of wood were placed in paper bags, enclosed in plastic and placed in cold storage ($<5^{o}C$) until detailed examination could be undertaken.

Just prior to examination, the bark was removed from the sapwood surface and fungi observed on both the wood surface and inner face of the bark were isolated aseptically. A small piece of sterile agar borne at the tip of a sterilized inoculating needle was touched to a spore mass either at the tip of a perithecial neck or being produced from a suspected anamorphic state. The piece of agar, now bearing spores, was placed in a petri dish containing either corn-meal agar (Gibco Diagnostics, Madison, Wisconsin) or 2% malt-extract agar (Johnston and Booth, 1983); both agars contained 1 mg/L of penicillin-g (Sigma Chemical Co., St. Louis, Missouri) and 1 mg/L of streptomycin sulphate (Sigma Chemical Co., St.Louis, Missouri). Plates so inoculated were incubated in a darkened growth chamber at $20^{o}C$ for

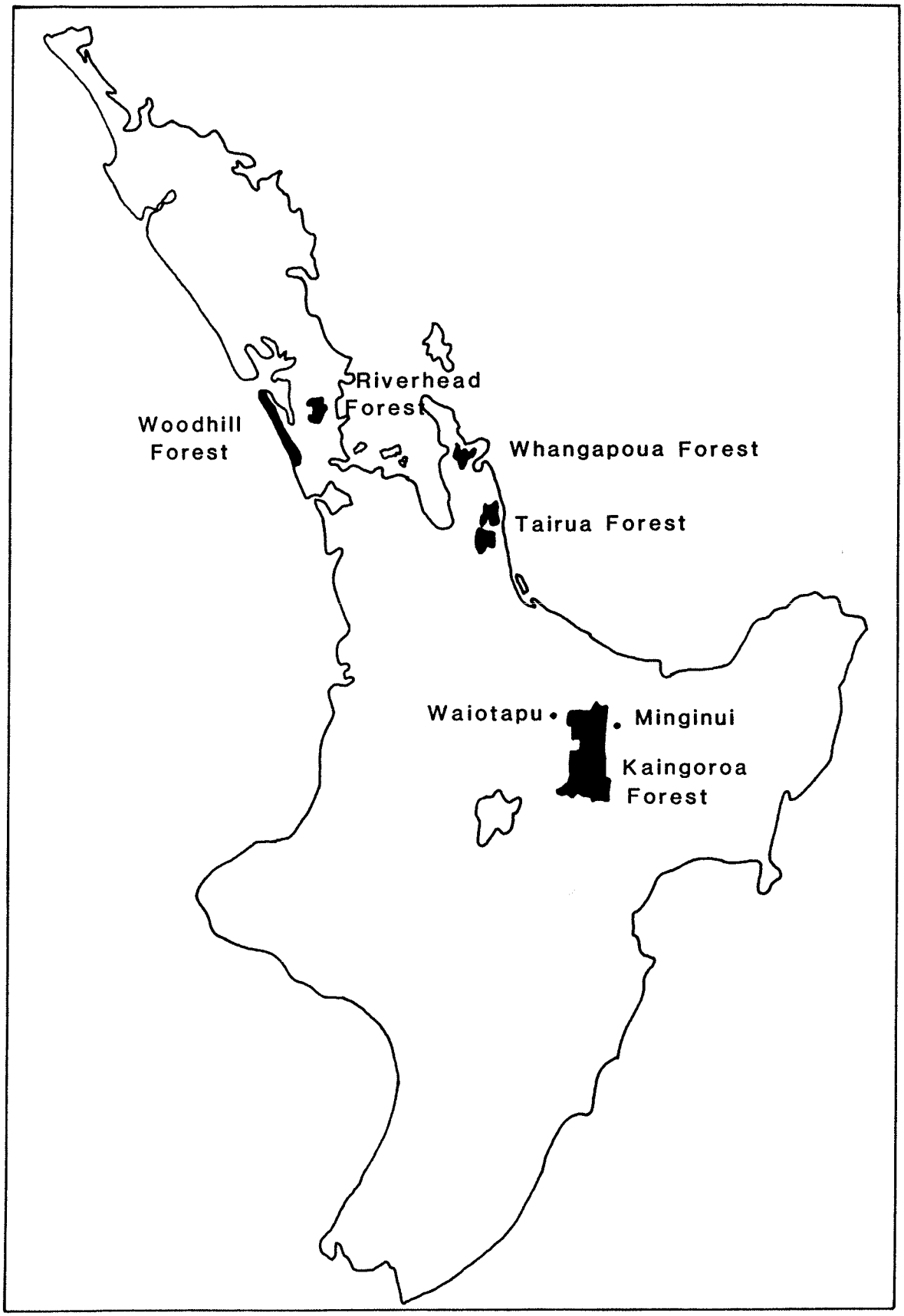


Figure 5. Collecting sites on the North Island of New Zealand

two to three days. Figure 6 illustrates how all such isolates were handled from isolation to the induction of the teleomorphic state and its identification.

When it was determined that each isolate was uncontaminated, stock cultures were made up in triplicate on 2% malt-extract agar slants and, after an incubation period of two to three weeks at 20°C, were stored at 9°C. These were later used as a source of inoculum to induce staining in wood, as well as for induction of perithecial production under various treatments.

Both Pinus and Picea spp. native to Manitoba were used to study staining by the fungi that were isolated. Small trees with basal diameters of 5 to 10 cm were cut and side branches removed. The stems were then cut into discs approximately 1.5 cm thick which were placed in air-tight plastic bags and frozen until used. When required, discs were placed singly into deep pyrex dishes (Corning 3250 storage dishes, Corning Glass Works, Corning, New York), each dish half filled with distilled water and autoclaved for 20 minutes at 15 p.s.i., on two successive days. On the third day, the water was removed and molten 4% water agar was poured into each deep dish until the level of the agar was just below the cut surface of the disc. When the agar hardened, the deep dishes were placed in the autoclave and sterilized for a third time. Afterwards, the discs were inoculated singly, each fungal culture onto one pine and one spruce disc, and incubated in a growth chamber

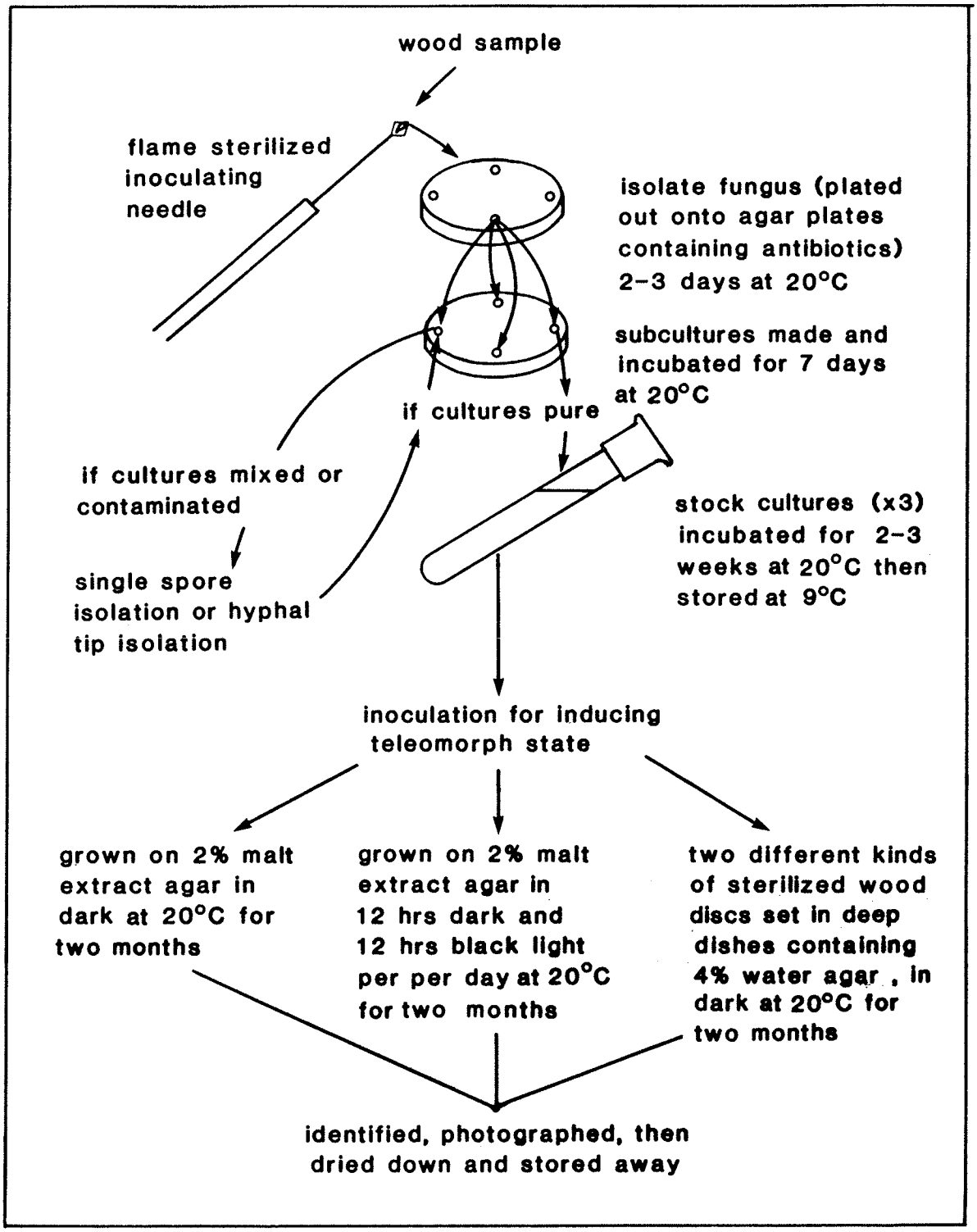


Figure 6. Steps in isolating fungi from wood until induction of teleomorphic state in culture (refer to text).

in the dark at 20°C for 60 days. These discs were removed, checked for perithecial formation, dried down and cut in half to observe the amount of staining, if any, that had taken place.

The treatments undertaken to induce perithecial formation were of two months duration at 20°C and were as follows: (1) growth on 2% malt-extract agar in continuous dark; (2) same agar, but cultures exposed to alternating 12 hour periods of dark and black light (General Electric and Sylvania 22 inch fluorescent tubes) supplemented with white light (Sylvania and Vita-Lite cool white 22 inch tubes); and (3) growth on sterilized wood discs in continuous darkness. If these failed, then those cultures which were likely to be the anamorphic states of the genus Ceratocystis were inoculated onto the medium described by Marshall et al. (1982) but modified so that the vitamins thiamine, biotin and pyridoxine were added. These test cultures were also grown in association with Gliocladium roseum (Link) Bainier, as it had been observed that G. roseum stimulated perithecial production of Ceratocystiopsis falcata (Wright & Cain) Upadh. In addition, they were grown on 2% malt-extract agar at cool temperatures (9°C) in the dark.

During incubation, each fungus was tentatively identified to anamorphic genus using taxonomic keys such as von Arx (1974), Barron (1968), Carmichael et al. (1980), Ellis (1971, 1976), and

Sutton (1980). Then, when the incubation periods were over, the isolates were examined for the presence of perithecia; if present these, along with the anamorphs, were identified to species. Those anamorphic fungi which caused staining in wood were also identified to species. Only rarely were fungi which neither stained nor belonged in the genus Ceratocystis identified to species since the emphasis of this research was on both the Ophiostomataceae and wood staining fungi.

Photographs were taken of the important taxonomic features of all representatives of the Ophiostomataceae and the wood staining fungi which were mounted in Melzer's solution without iodine. Either phase contrast microscopy using a Zeiss Photomicroscope II or Nomarski interference contrast microscopy using a Leitz Ortholux II was employed using Kodak Panatomic X film. The film was developed in Kodak D-76 developer and prints were made on Kodak Polycontrast-F photographic paper using Kodak D-72 developer and standard procedures.

RESULTS AND DISCUSSION

(a) Ophiostomataceae

1. Ceratocystiopsis falcata (Wright & Cain) Upadhyay, Monogr. Ceratocystis and Ceratocystiopsis, p.125, 1981 Plate I, Figs. a-e.

≡ Ceratocystis falcata Wright & Cain, Can. J. Bot. 39: 1226, 1961.

ANAMORPHS: Chalara spp.

Colonies attaining a diameter of 14-20 mm in 12 days at 20°C on 2% malt agar; appressed, hyaline, but becoming brown in the immediate vicinity of the developing perithecia[2]. First anamorphic state with hyaline to pale brown conidiophores; thin-walled, simple to occasionally branching, smooth, septate, up to 70 µm long (including phialides) and 2.3-5.7 µm wide. Conidiogenous cells monophialidic, integrated, terminal, determinate; 18-37.5 µm long and 2.25-4.8 µm wide at their broadest part and 1.0-2.4 µm wide at their tips. Conidia cylindrical to occasionally

[2]Names for colours derived from Munsell Colour Company. 1973. Munsell Soil Colour Chart. Kollmorgen Corp., Baltimore, Md.

oblong with obtuse to truncate ends; hyaline; one-celled; endogenous; catenate; 4.0-8.6 x 1.3-2.1 μm. Second anamorphic state with golden-brown to dark-brown conidiophores; thick-walled; simple to occasionally branching; up to 95 μm long (including phialides) and 5.2-7.4 μm wide; smooth, septate. Conidiogenous cells monophialidic; integrated, terminal, determinate; 26.2-40.4 μm long and 5.7-7.5 μm wide at their broadest part and 3.3-4.3 μm wide at their tips. Conidia cylindrical to occasionally oblong with obtuse to truncate ends; hyaline to rarely very pale brown; one-celled, endogenous; catenate; 4.3-10 x 2.4-4.5 μm.

Perithecia developing superficially on both the mycelium in culture and on the natural substrate. Bases globose to flattened dorsi-ventrally and ornamented with dark-brown hyphal elements 95-155 μm by 88-145 μm [50-80 μm (Wright and Cain, 1961); (47-) 56-93.5(-100) μm (Upadhyay, 1981)]. Necks short, conical, dark-brown to black but much lighter at the apex; 20-40 μm high and 30-50 μm wide at the base; terminating in pale-brown to hyaline convergent ostiolar hyphae up to 15 μm long and 1.0-1.5 μm wide (projecting rounded cells according to Wright and Cain, 1961; laterally fused filaments according to Upadhyay, 1981). Asci evanescent; 8-spored clavate, fusiform-elliptic, to clavate; (18-)20-28(-32) x 2.4-5 μm [20-28 x 2.4-3.2 μm (Wright and Cain, 1961); (18-)22-30(-35) x 2.5-5.5(-7) μm (Upadhyay, 1981)]. Ascospores hyaline; fusiform in plan view[3], falcate in side view,

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[3]Ascospore views as defined by Olchowecki and Reid (1974).

end view not seen; 20.0-33.2 x 1.2-2.0 μm; emerging from the ostiole in long, thread-like cirrhi.

NEW ZEALAND HOSTS: Larix sp., Pinus radiata D. Don

CULTURES ISOLATED: R 23 (d) isolated from Larix sp., Waiotapu Forest; R 41 isolated from Pinus radiata, Woodhill Forest; R 98 isolated from Pinus radiata, Whangapoua Forest.

Ceratocystis falcata was described by Wright and Cain (1961), but they made no mention of an anamorphic state. This species was described as new because they felt that its small perithecia with short conical necks were unlike those of any other species of the genus, except for Ceratocystis minuta (Siem.) Hunt; however, the size and septation of the ascospores of C. falcata readily distinguished it from C. minuta. Subsequently Upadhyay (1981) transferred C. falcata to the genus Ceratocystiopsis. Rayner and Hudson (1977) noted from their examination of British isolates that an anamorph was produced; it was a typical Chalara sp.

Isolates of this fungus obtained from New Zealand possess the major characters of C. falcata; however, a number of minor discrepancies were noted as described below.

The occurrence of convergent ostiolar hyphae on the perithecia of the New Zealand isolates of this fungus is distinctive to that noted by previous authors. Wright and Cain (1961) do not mention ostiolar hyphae; they merely state that slightly projecting, rounded, hyaline cells occur at the ostiolar apex. Rayner and Hudson (1977) assert that ostiolar hyphae are absent, while

Plate I

Ceratocystiopsis falcata (Wright & Cain) Upadhyay

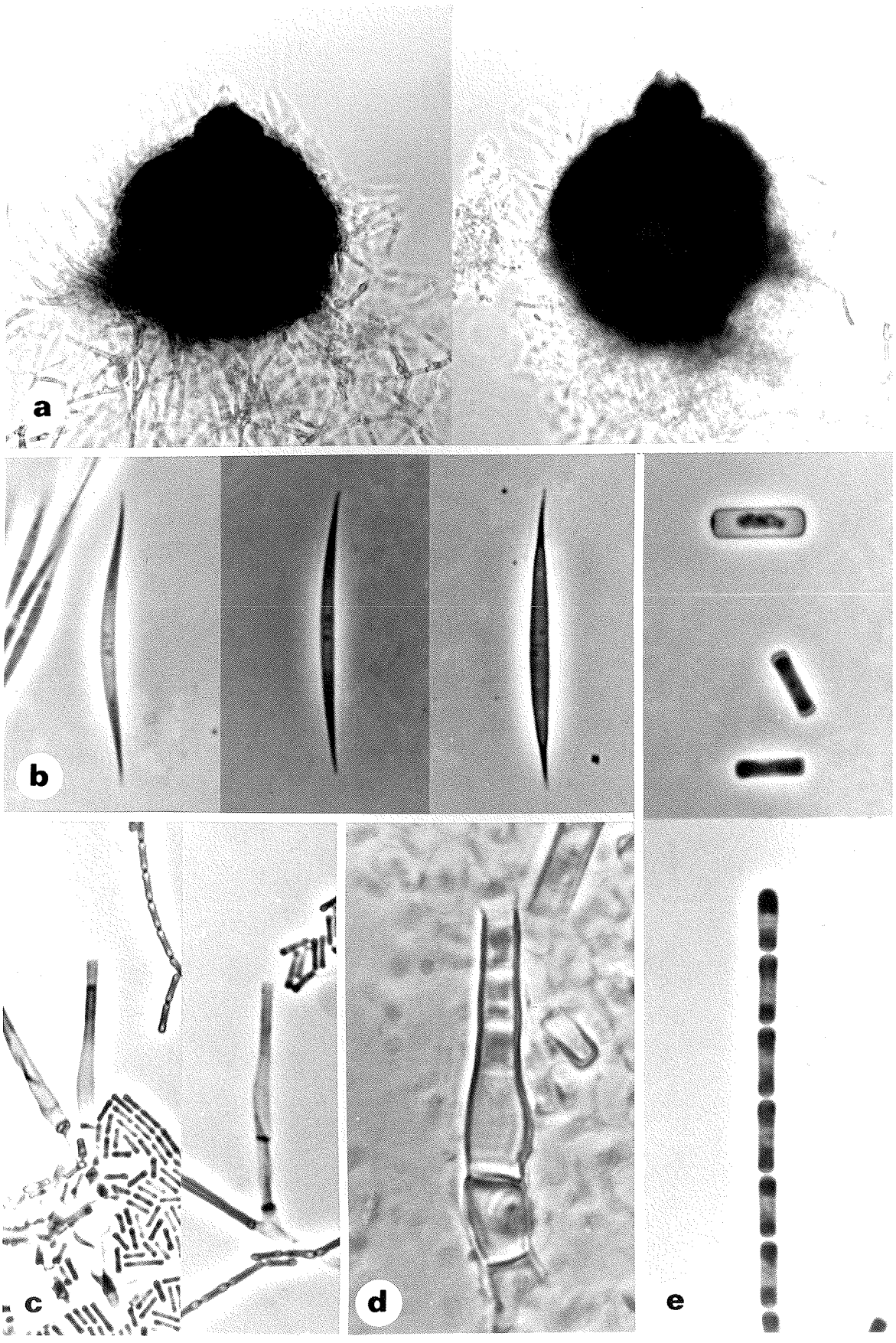
Fig. a. Perithecia; note short, converging ostiolar hyphae (X310).

Fig. b. Ascospores; note septum (X2000).

Fig. c. Phialides of smaller *Chalara* state (X875).

Fig. d. Phialide of larger *Chalara* state associated with perithecia (X2000).

Fig. e. Conidia; large (top), small (middle), and small conidia in a chain (bottom) (X2000).


a
b
c
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e

Upadhyay (1981) believed that the ostiolar hyphae were laterally fused filaments which converged to form a narrow opening which is slightly protruding beyond the apical part of the neck. The convergent ostiolar hyphae are not always obvious and therefore it is not surprising that the above workers reached their respective conclusions.

Another difference appears to be the size of the perithecia which are much larger in the isolates from New Zealand than those described by other workers; 95-155 µm in diameter in this study as compared with 50-80 µm (Wright and Cain, 1961), 60-85 µm (Rayner and Hudson, 1977), and 47-100 µm (Upadhyay, 1981). However, the perithecia of the New Zealand isolates are often slightly broader than high and this corresponds to Wright and Cain's (1961) observations.

While the ascospores as described above (Fig.b, Plate I) generally agree well with the descriptions provided by previous workers, there is a problem with respect to whether a sheath is, in fact, present. Neither Wright and Cain (1961) nor Rayner and Hudson (1977) mention the presence of a sheath. Upadhyay (1981), however, states the ascospores are falcate with obtuse ends in side view and fusiform in plan view and are enclosed in a hyaline gelatinous sheath which appears falcate with attenuated ends in side views and acicular in plan view; he clearly implies the spore ends are obtuse, the falcate appearance being due to the sheath. While the ascospores from the New Zealand isolates do seem to agree with Uphadyay's description, it is doubtful that a sheath is truly present.

Rounding off of the protoplasm near the ends of the ascospores may give the illusion that the ends of the spore proper are part of a sheath which extends beyond either end of the ascospores; probably it is simply the protoplast that Upadhyay is considering as the spore proper. However, only through the use of transmission electron microscopy will the question be settled.

Rayner and Hudson (1977) and Upadhyay (1981) make mention of only one Chalara state; this study has uncovered a second, larger spored Chalara state as well. The larger Chalara anamorph is only found associated with the perithecia. While there is some overlap in measurements of these two phialidic states, they are clearly distinct; differences include thickness of the cell walls, colour, size of the conidia and width at the apex of the phialides.

In vitro, C. falcata grows very slowly and only produces the smaller anamorphic state. However, when grown with Gliocladium roseum or when filtered extracts of G. roseum were added to the media, C. falcata would produce ascocarps (see section B, Perithecial Induction in Ceratocystiopsis falcata).

de Hoog and Scheffer (1984) suggested that C. falcata is very similar to species of the genus Pyxidiophora Bref. & Tav. because of its ascospore morphology and its Chalara imperfect state. While illustrations in Lundqvist (1980) do indicate superficial similarities between C. falcata and Pyxidiophora nyctalidis Bref.

& Tav. (1891), Pyxidiophora spp., unlike C. falcata have ascospores with a pigmented spot. Further, Pyxidiophora species appear to be chiefly coprophilous while C. falcata occurs as a saprophyte only on woody hosts.

2. Ceratocystis coronata Olchow. & Reid, Can. J. Bot. 52: 1705, 1974 Plate II, Figs. a-g.

ANAMORPH: Sporothrix sp.

Colonies attaining a diameter of 30-34 mm in 12 days at 20°C on 2% malt agar; flocculose to funiculose; at first hyaline, becoming white as the colony enlarges. Conidiophores mononematous; micronematous to semi-macronematous. Conidiogenous cells polyblastic, integrated, terminal, determinate; denticulate. Conidia produced sympodially upon the denticles; hyaline; one-celled; clavate and curved to occasionally fusiform; 4.0-10.0 x 0.8-1.5 µm (2.0-8.0 x 1.0-2.5 µm, Olchowecki and Reid, 1974). Ramoconidia formed occasionally; 7.0-10.0 x 1.5-2.0 µm.

Perithecia superficial on the natural substrate, and developing superficially on substrates in culture within two weeks. Bases globose; dark-brown to black, and ornamented with dark-brown hyphal elements; 100-175 µm in diameter [45-110(-160) µm, Olchowecki and Reid]. Perithecial necks black; straight, curved, or geniculate; becoming annulate at times because of percurrent

proliferations through a previous neck apex; hyphal fringes at the annuli; 355-1,930 µm long [100-450(-600) µm, Olchowecki and Reid], 20-40 µm wide at the base, and 7.5-10 µm wide at the tip (just below the apex). Ostiolar hyphae present at the neck apex; hyaline, septate, divergent; 16-65 µm long and 1.5-2.1 µm wide at the base. Ascospores hyaline, one-celled; allantoid in side view, oblong to cylindrical with obtuse ends in plan view, globose to spherical in end view; 3.0-5.2 x 0.8-1.3 µm; sheath lacking; emerging from the ostiole and forming a spore ball at the tip.
NEW ZEALAND HOSTS: Eucalyptus sp., Pinus nigra Arnold, and Pinus radiata D. Don.
CULTURES ISOLATED: R 42 isolated from Pinus radiata, Woodhill Forest; R 168 isolated from Pinus nigra, Kaingoroa Forest; 181 (bi) isolated from Eucalyptus sp., Waiotapu Forest; 181 (ci) isolated from Eucalyptus sp., 181 (f) isolated from Eucalyptus sp., Waiotapu Forest; 181 (g) isolated from Eucalyptus sp., Waiotapu Forest; R 199 (c) isolated from Eucalyptus sp., Riverhead Forest..

A number of isolates of a Ceratocystis sp. obtained during this study appear best disposed under the name of Ceratocystis coronata. However, differences exist between the Manitoba isolates of Olchowecki and Reid and those obtained from New Zealand.

The perithecial necks of the New Zealand isolates are much longer than those described by Olchowecki and Reid (1974) and the former also exhibit annulations, a feature not mentioned by Olchowecki and Reid in their discussion. Annulations on the necks

Plate II

Ceratocystis coronata Olchowecki & Reid

Fig. a. Perithecia; note presence of annulations on neck of perithecium on right (X100).

Fig. b. Annulations on perithecial neck (X250).

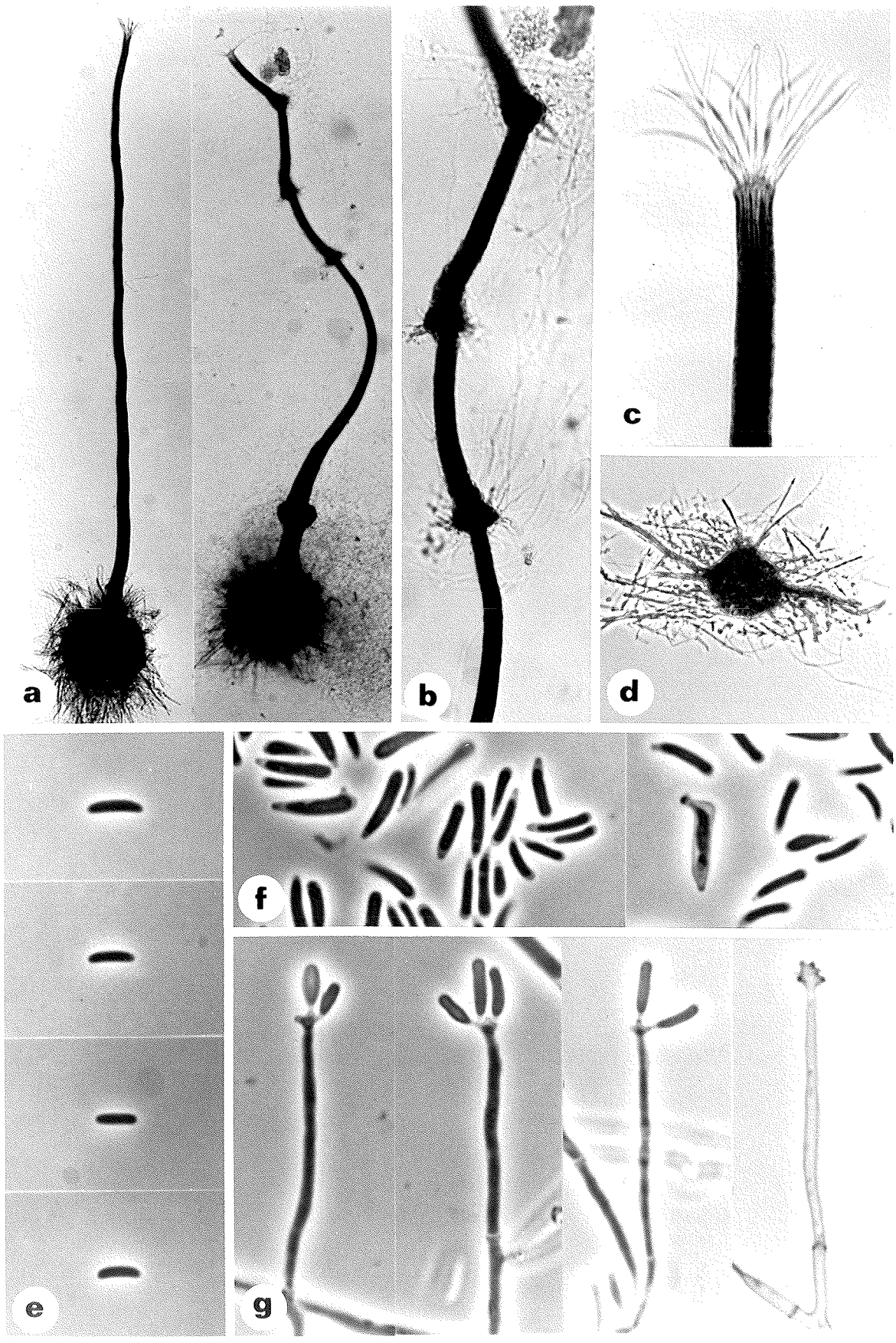
Fig. c. Ostiolar hyphae (X620).

Fig. d. Protoperithecium (X250).

Fig. e. Ascospores; side and plan views (X1800).

Fig. f. Conidia; note ramoconidium (X1800).

Fig. g. *Sporothrix* state (X1800).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of this species should not be viewed as being unusual as Griffin (1968) points out that many species of Ceratocystis exhibit annulations if grown under suitable conditions.

When Ceratocystis spp. are grown in vitro, the presence of protoperithecia usually indicates that the species is heterothallic, as demonstrated by Gregor (1932) for Ceratocystis pluriannulata and by Mittman (1932) for Ceratocystis pilifera (Fr.) C. Moreau. Four of the seven New Zealand isolates produced mature perithecia in culture while the other three only produced protoperithecia; these three were all derived from the same collection as a fourth isolate which produced mature perithecia. Making crosses in all combinations of the three protoperithecial isolates did not yield mature perithecia, indicating that these cultures were of the same mating type.

Upadhyay (1981) reduced C. coronata to synonymy with Ceratocystis tenella Davids. This is felt to be incorrect for a number of reasons. For example, ascospore morphology is one of the most reliable characters in this genus, varying very little under various environmental conditions. C. tenella was described as having ascospores which were orange-section shaped in side view while C. coronata has ascospores which are allantoid in side view. Both species have Sporothrix states but C. tenella was described as producing few conidia whereas C. coronata produces abundant conidia from prominent denticles of the conidiogenous cell. C. tenella was described as developing some dark colour in patches in

the colony while C. coronata remains white. Based on the above it is felt that C. coronata should remain as a discrete species.

3. Ceratocystis ips (Rumb.) C. Moreau, Rev. Mycol. Suppl. Colonial, 17: 22, 1952 Plate III, Figs. a-e and Plate IV, Figs. a-d.

≡ Ceratostomella ips Rumb., J. Agric. Res. 43: 864, 1931.

≡ Ophiostoma ips (Rumb.) Nannf. Sv. Skogsvardsf. Tidskr. 32: 408, 1934.

≡ Grosmannia ips (Rumb.) Goid., R. Staz, Pat. Veg. Bol., Rome, n.s. 16: 51, 1936.

= Ceratocystis montia (Rumb.) Hunt. Lloydia 19: 45, 1956 fide Upadhyay (1981).

≡ Ceratostomella montium Rumb., J. Agric. Res. 62: 597, 1941.

≡ Ophiostoma montium (Rumb.) Arx, Antonie van Leeuwenhoek 18: 211, 1952.

= Ceratocystis adjuncta Davids., Mycologia 70: 35, 1978 fide Upadhyay (1981).

SYNANAMORPHS: (1) Graphilbum sp. fide Upadhyay (1981), (2) Hyalorhinocladiella sp., and (3) Acremonium sp.

Colonies attaining a diameter of 68-84 mm in 9 days at 20^{o}C on 2% malt extract agar; appressed; colour variable; at first hyaline, but becoming either light yellowish-brown, very

pale-brown, olive-brown or very dark-grey depending on the isolate; on occasion hyphal elements appear to be regularly constricted at the septa, and the pseudocells then resemble acropetalous chains of blastospores. Graphilbum synanamorph: Conidiophores mononematous and macronematous, synnematous, sporodochial, or absent; synnematal stipe hyaline to dark-coloured, sporodochial conidiophores produced in an inverted conical manner from a small aggregation of prostrate hyphae embedded in the agar. Conidiogenous cells phialidic, integrated, terminal, percurrent, cylindric to rarely slightly lageniform. Conidia enteroblastic, one-celled, oblong with obtuse to truncate ends; 4.0-16.5 x 1.0-3.0 µm, produced in slimy heads. Hyalorhinocladiella synanamorph: Conidiophores mononematous, semi-macronematous, and often with an inflated basal cell; hyaline. Conidiogenous cells hyaline; polyblastic, terminal, integrated, tapering towards the apex, sympodial. Conidia hyaline, one-celled; ellipsoidal or ovoid to turbinate; 2.0-6.0 x 1.5-3.0 µm. Acremonium synanamorph: Rare, but when present, represented by cylindrical phialides 9.0-20.0 µm long and 2.2-3.2 µm wide; hyaline. Phialospores hyaline; ellipsoid to spherical; 3.0-5.0 x 1.5-3.5 µm.

Chlamydospores, when present, light brown; globose; solitary; terminal or intercalary; 7.5-18.0 µm in diameter.

Perithecia developing either superficially on the mycelium or immersed (often deeply) in the agar, within 30 days from inoculation. Bases globose; light-brown to black; 160-415 µm in diameter; with or without dark-brown ornamenting hyphal elements.

Necks straight or curved; tapering towards the tip; black, but often paler at the apex; 195 μm to 2.5 mm in length (up to 1200 μm long, Upadhyay, 1981); 25-65 μm wide at their bases and 10-35 μm wide at their tips. Ostiolar hyphae absent. Ascosopores hyaline; one-celled; oblong in side and plan view, spherical in end view; possessing a hyaline sheath which appears in side and plan view slightly concave in the long axis of the spore, convex in the short axis but projecting at the corners (most authors describe the sheath as quadrangular, e.g. Upadhyay, 1981); quadrangular in end view, but with slightly concave surfaces, 3.0-4.5 x 1.5-3.0 μm; emerging from the ostiole as spiral-forming cirrhi.
NEW ZEALAND HOSTS: Pinus elliotii Engelm. and Pinus radiata D. Don.
CULTURES ISOLATED: R 57 (b) isolated from Pinus radiata, Tairua Forest; R 58 isolated from Pinus radiata, Tairua Forest; R 59 (b) isolated from Pinus radiata, Tairua Forest; R 70 (a) isolated from Pinus elliotii, Tairua Forest; R 70 (bi) isolated from Pinus elliotii, Tairua Forest; R 77 (bi) isolated from Pinus radiata, Tairua Forest; R 83 (a) isolated from Pinus radiata, Tairua Forest; 83 (gii) isolated from Pinus radiata, Tairua Forest; R 84 (d) isolated from Pinus radiata, Tairua Forest; R 87 (a) isolated from Pinus radiata, Tairua Forest.

Ceratocystis ips was the most pleomorphic of all fungi isolated during this study. It has three separate distinct anamorphs, which by themselves indicate a high degree of pleomorphism,

Plate III

Ceratocystis ips (Rumbold) C. Moreau

Fig. a. Perithecia; note appendages on base of perithecium on left side compared with appendage-free base of perithecium on right side (X90).

Fig. b. Ascospores; side, plan and end views; third from bottom without sheath (X1800).

Fig. c. Conidia of *Graphilbum* state (X1500).

Fig. d. Sporodochium of *Graphilbum* state (X235).

Fig. e. Conidiogenous cell of *Graphilbum* state; note percurrent proliferation (X1800).

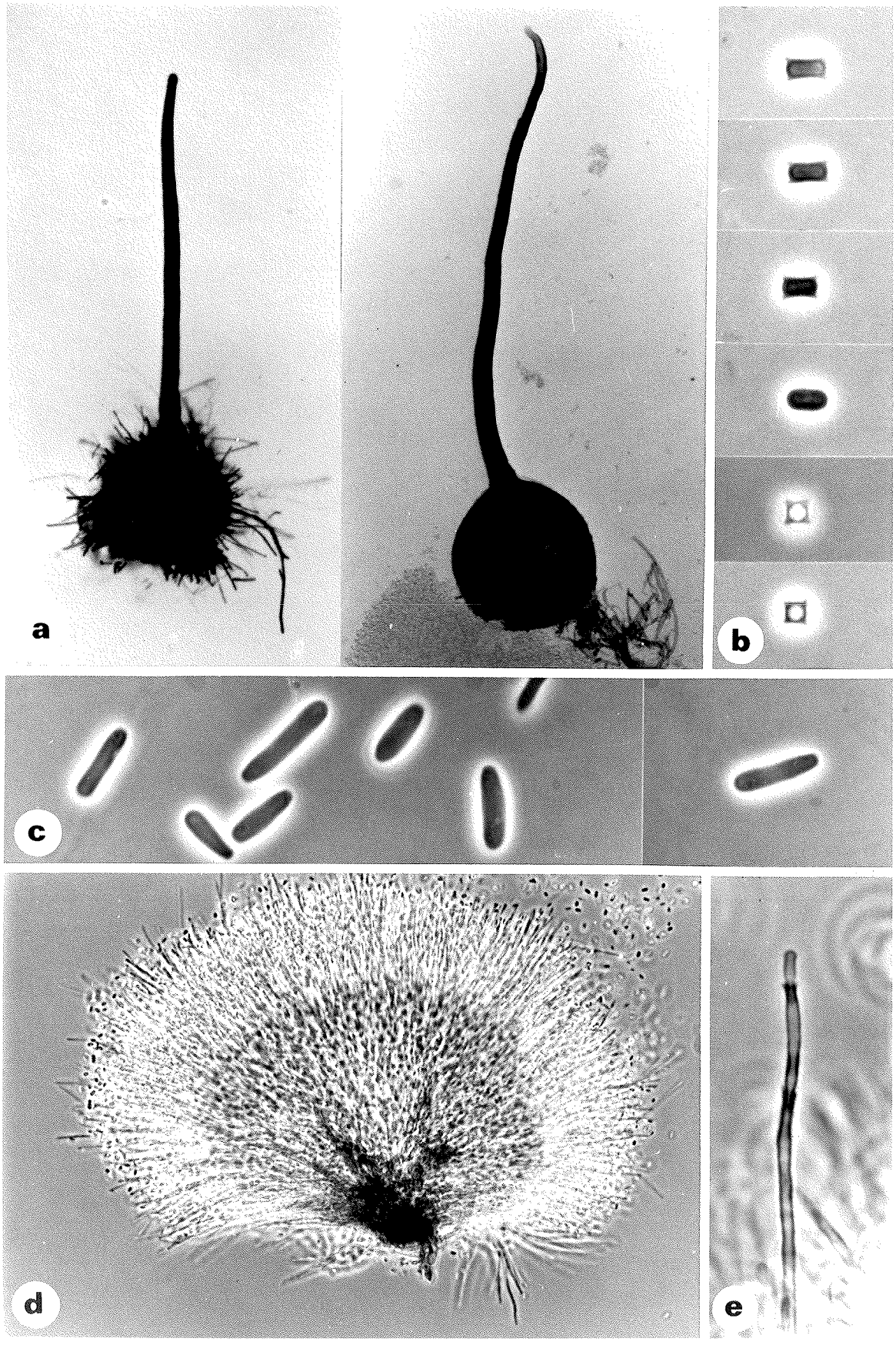
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Plate IV

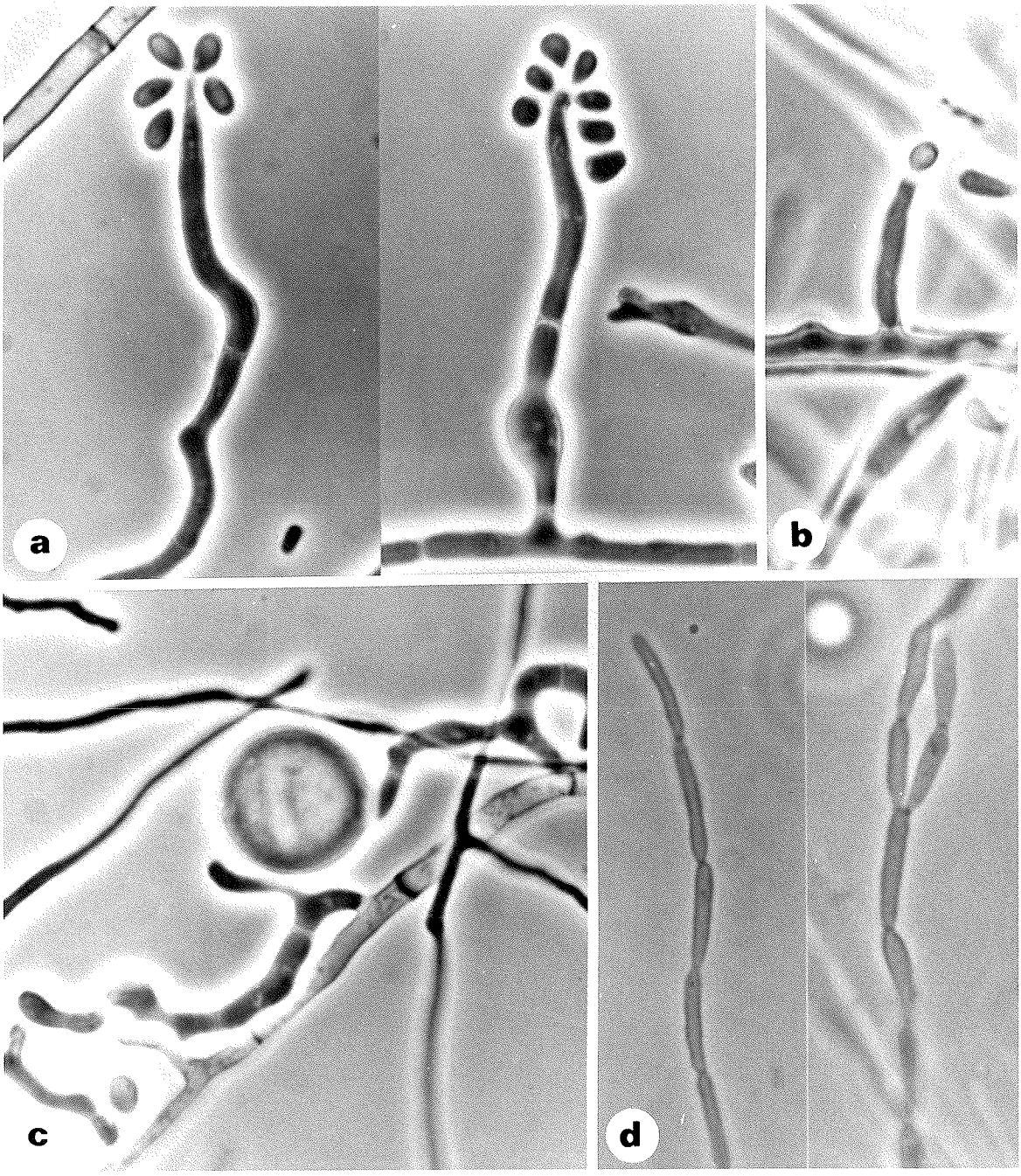
Ceratocystis ips (Rumbold) C. Moreau

Fig. a. *Hyalorhinocladiella* state (X1800).

Fig. b. *Acremonium* state (X1800).

Fig. c. Chlamydospore (X1800).

Fig. d. Constricted hyphae (X1800).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in addition to perithecia which are often variable in form. Indeed as Upadhyay (1981) suggests, the ascospore characters are the most reliable features one can employ in the identification of C. ips.

In one isolate obtained, the hyphae became constricted at the septa (Fig.d, Plate IV), and then appeared similar to chains of blastospores which one would find in the genus Cladosporium Link ex Fr. or Hyalodendron. It is possible aging of the colony was involved in triggering this phenomenon since transfers from such cultures produced normal hyphae in the fresh cultures. Nisikado and Yamauti (1933) who studied Japanese isolates of this fungus reported, and illustrated, that if grown on rice-jelly agar, the hyphal cells produced budding branches which were roundish, spherical or pear-shaped and Rumbold (1941) noted older hyphae in C. montia (C. ips fide Upadhyay, 1981) consisted of rows of short, slightly globular cells.

Upadhyay (1981) assigned the synnematal anamorph to the genus Graphilbum; however, it is really difficult to assign this anamorphic state to any single genus and it is questionable whether generic names should be applied to any asexual form of the fungus. It is extremely variable in its habit, sometimes appearing similar to a member of the Leptographium complex, sometimes appearing as a true Graphilbum and at other times producing conidiophores attached to almost non-existent synnemata. When formed in the latter way, the anamorphic fructification is very

similar to a sporodochium attached to the substrate by basal cells. Olchowecki and Reid (1974) report a similar situation in Ceratocystis cainii Olchow. & Reid where Graphium-like synnemata are produced on wood but not in agar culture; here the conidiophores developed from a fan-shaped structure which itself was derived from a single basal cell. Nisikado and Yamauti (1933) found that in their isolates of C. ips the conidia were never produced on a Graphium-like conidiophore, although references are made in their paper to conidia being produced from sporodochia on certain types of agar media.

Webb (1945) isolated a fungus she identified as Leptographium lundbergii from two Eucalyptus spp. and a Nothofagus sp. in Australia. From her illustrations and written account it is clear that her fungus produced a range of different forms very similar in range of appearance to that which one would expect to see from the synanamorphs of C. ips. In addition, she also found what she claimed was the possible perithecial state of her fungus; the perithecia were deeply embedded into the wood, possessed bristles at the tips of the necks and produced ascospores which were similar in size and shape to those of C. ips. During the present study it was found that C. ips produced perithecia which were often deeply embedded in the agar medium, but ostiolar hyphae were never noted. However Nisikado and Yamauti (1933) illustrated that in some cases bristles occurred at the apex of the perithecial necks. It is quite likely that Webb's

organism was not Leptographium lundbergii at all but simply the Graphilbum pleomorphic state of C. ips.

On occasion, isolates of C. ips examined during this study produced perithecia which were light brown in colour and had very smooth bases; these were unlike the normally found dark-coloured perithecia whose bases were covered with perithecial hairs. However the ascospores were still typical of C. ips, and this observation reinforces the earlier comment that ascospore characteristics are the only constant character in identification of this species.

In culture, large solitary chlamydospores were observed, but only when the fungus was grown onto glass slides. This is the only Ceratocystis species which produced chlamydospores in this study and this is a feature previously unreported for this species. Chlamydospore formation is a common characteristic of fungi when they are exposed to harsh environmental conditions, and growing mycelium onto glass slides could be considered, at least by the fungus, as exposure to a harsh environment

4. Ceratocystis novae-zelandiae sp. nov. Plate V, Figs. a-h.

SYNANAMORPHS: (1) Pesotum sp. and (2) Sporothrix-like to Hyalodendron-like conidial form.

Coloniae in agaro cum extracto malti (2%) adpressae-floccosae, plerumque prope perithecia evolventia caespitosae; post 12 dies fuscae, caespes myceliales albi remanentes. Synana-

morpha prima similis Pesoto raro visa; stipite 560-840 µm longo, 50-200 µm lato basi, 50-160 µm lato infra zonam conidiogenam; conidiis hyalinis, sympodialibus ellipsoideis vel ovoideis vel oblongis, 2.5-5.0 x 1.0-2.2 µm. Synanamorpha altera, aut similis Sporotrichi in denticulis conspicuis sympodulosporas, aut similis Hyalodendro in catenis acropetis conidia holoblastica efferens; conidiis hyalinis ellipsoideis vel fusiformibus 4.0-7.5 x 1.0-1.5 µm; ramoconidiis 8.0-16.0 x 1.0-3.0 µm.

Perithecia basibus globosis nigris, aut laevibus aut pilis fuscis ornatis, 125-170 µm diametro; collis ad 10 mm vel plus longis, 25-35 µm latis basi, 10-16 µm proxime infra apicem, saepe annulatis; hyphis ostiolaribus hyalinis, septatis, divergentibus, 20-35 µm longis, 2.0-2.5 µm latis basi. Ascosporae hyalinae, aseptatae, plerumque allantoideae sed raro a latere conspectae cum figura segmenti pomi citri, superne ovales, ab extremo conspectae circulares; 4.0-5.0 x 1.0-1.5 µm; vagina nulla. TYPUS: R 137 (a) sejunctus ex Podocarpus sp., ex sylva Minginuii, Nova Zelandia. J. Reid legit June 11, 1982.

Colonies attaining a diameter of 60 mm in 12 days at 20°C on 2% malt agar; appressed to floccose and often very tufted in patches where the perithecia develop; at first hyaline, becoming dark-brown to olive-brown, finally very dark greyish-brown; mycelial tufts where perithecia frequently develop remaining white. Pesotum synanamorph: infrequently observed; conidiophores macronematous, synnematous; synnemata with dark-coloured stipes and lighter apices

where the conidiogenous cells are borne on penicillate branches. Stipe 560-840 µm in length, 50-200 µm wide at the base, 50-160 µm wide immediately below the conidiogenous zone. Conidiogenous cells polyblastic, sympodial, terminal to rarely intercalary. Conidia hyaline; one-celled; ellipsoid to ovoid to oblong and slightly tapering to their point of attachment; 2.5-5.0 x 1.0-2.2 µm, produced in slimy heads. Sporothrix-like to Hyalodendron-like synanamorph: conidiophores mononematous; hyaline; micronematous to semi-macronematous. Conidiogenous cells polyblastic; integrated; sympodial. Sympodulospores produced on well-defined denticles in the Sporothrix form; in holoblastic acropetalous chains in the Hyalodendron form. Conidia hyaline; ellipsoidal to fusiform and tapering to their point of attachment; 4.0-7.5 x 1.0-1.5 µm. Ramoconidia 8.0-16.0 x 1.0-3.0 µm

Perithecia developing superficially on the mycelium within two weeks following inoculation. Bases globose; black; 125-170 µm in diameter; ornamented with dark hairs or smooth. Necks straight or curved; black in colour; up to 10 mm or more in length; 25-35 µm wide at the base and 10-16 µm wide at tip below apex. Annulations frequently forming along the neck due to percurrent proliferations through previously existing ostioles. Ostiolar hyphae hyaline, septate, divergent; 20-35 µm long and 2.0-2.5 µm wide at base. Ascospores hyaline; one-celled, chiefly allantoid to occasionally orange section-shaped in side view, oval in plan view and spherical in end view; 4.0-5.0 x 1.0-1.5 µm; sheath

absent; emerging from the ostiole and forming a spore ball at the tip.

TYPE: R 137 (a) isolated from Podocarpus sp., Minginui Forest, New Zealand collected by J. Reid June 11th, 1982.

NEW ZEALAND HOSTS: Pinus radiata D. Don, Podocarpus spicatus R. Br. ex Mirbel, Podocarpus sp., Pseudotsuga menziesii (Mirb.) Franco.

CULTURES ISOLATED: R 15 (d) isolated from Pseudotsuga menziesii, Kaingoroa Forest; R 137 (a) isolated from Podocarpus sp., Minginui Forest; R 147 (d) isolated from Podocarpus spicatus, Minginui Forest; R 147 (d)' isolated from Podocarpus spicatus, Minginui Forest; R 163 (b) isolated from Pinus radiata, Kaingoroa Forest.

The only other Ceratocystis sp. with which Ceratocystis novae-zelandiae could be confused is Ceratocystis nothofagi Butin, in Butin and Aquilar (1984). Despite some similarities, e.g. extremely long necks and formation of tufts of white mycelium, there are significant differences between these two species. Ascocarps of C. novae-zelandiae readily form in vitro, while those of C. nothofagi only form on autoclaved wood chips; the necks of C. novae-zelandiae are consistently long when grown in vitro while those of C. nothofagi are considerably shorter in vitro than in the natural habitat; C. nothofagi has very prominent orange-section shaped ascospores while those of C. novae-zelandiae are more allantoid in shape; C. novae-zelandiae produces a Pesotum state in addition to the

Plate V

Ceratocystis novae-zelandiae sp. nov.

Fig. a. Perithecium; note the extremely long neck in relation to the size of the venter (X13).

Fig. b. Protoperithecium (X250).

Fig. c. Apex of perithecial neck with ostiolar hyphae. An annulation created by a percurrent proliferation through a previously existing neck apex, where ostiolar hyphae are still located, can be seen (X250).

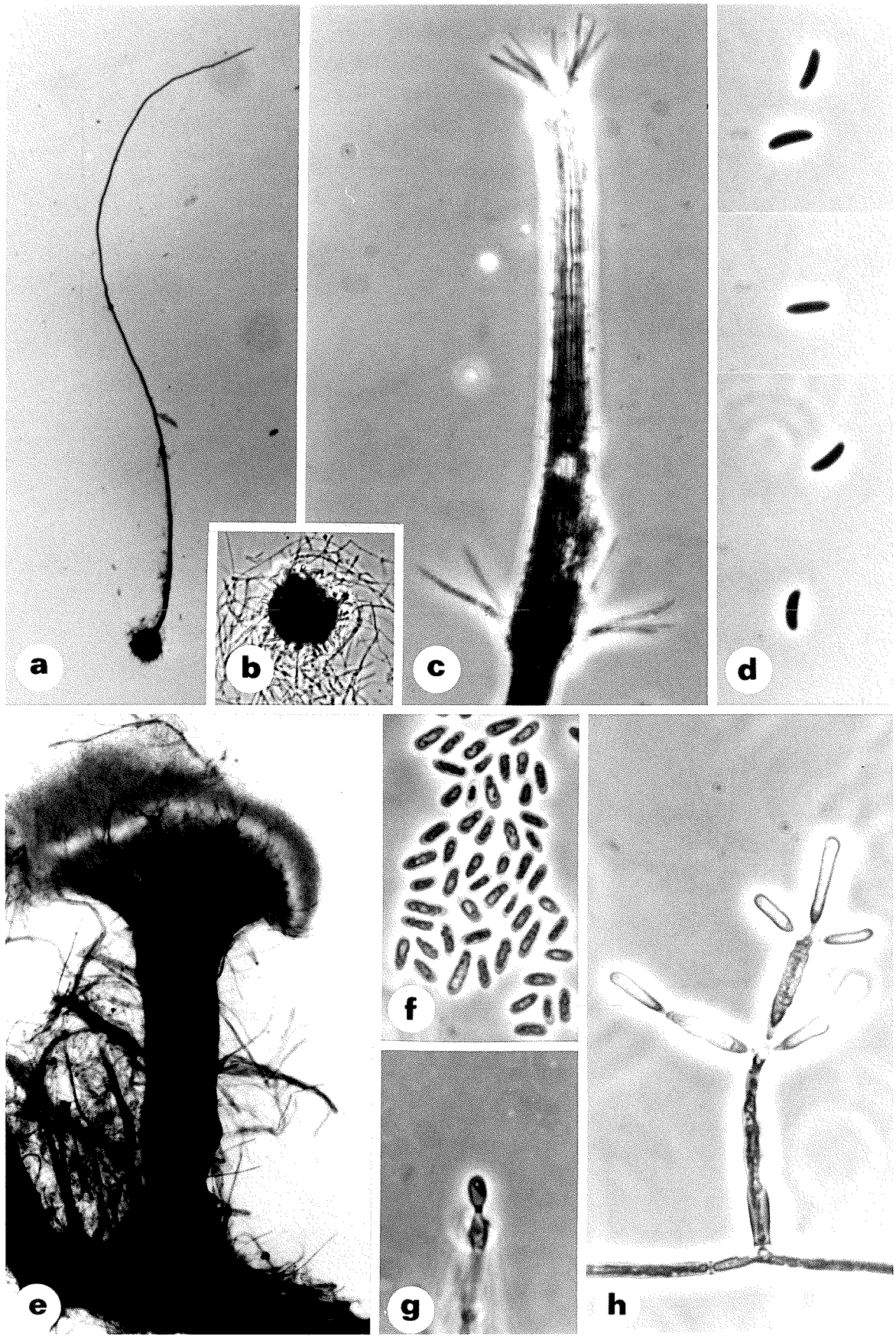
Fig. d. Ascospores (X1550).

Fig. e. A synnema of the *Pesotum* state (X100).

Fig. f. Conidia of *Pesotum* state (X1550).

Fig. g. Sympodial conidiogenous cell with conidium of *Pesotum* state (X1550).

Fig. h. Mononematous anamorphic state; when appearing *Hyalodendron*-like (X1550).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Sporothrix-like to Hyalodendron-like anamorph while C. nothofagi has only a Sporothrix anamorphic state; and, finally, C. novae-zelandiae grows much faster than C. nothofagi.

The presence of protoperithecia, as has been mentioned for other species isolated in this study, indicates the probability of heterothallism in this species.

The Pesotum state rarely occurs in vitro. In fact it can be easily overlooked. This phenomenon has been noticed previously in Ceratocystis perfecta Davids., where de Hoog (1974) observed a synnematous state while Davidson (1958) and Upadhyay (1981) did not.

5. Ceratocystis piceae (Münch) Bakshi, Trans. Brit. mycol. Soc. 33: 113, 1950 Plate VI, Figs. a-e.

≡ Ceratostomella piceae Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 547, 1907.

≡ Ophiostoma piceae (Münch) H. & P. Sydow, Annls mycol. 17: 43, 1919.

= Ceratocystis querci (Georgew.) C. Moreau, Rev. Mycol. Suppl. Colonial, 17: 22, 1952 fide Hunt (1956).

≡ Ceratostomella querci Georgew., Acad. Sci. Compt. Rend. (Paris) 183: 759, 1926.

= Ceratocystis fagi (Loos) C. Moreau, Rev. Mycol. Suppl. Colonial, 17: 22, 1952 fide de Hoog (1974).

≡ Ceratostomella fagi Loos, Arch. F. Mikrobiol. 3: 376, 1932.

≡ Ophiostoma fagi (Loos) Nannf., Sv. Skogsvårdsf. Tidskr. 32: 408, 1934.

= Ceratocystis catoniana (Goid.) C. Moreau, Rev. Mycol. Suppl. Colonial, 17: 22, 1952 fide de Hoog (1974).

≡ Ceratostomella catoniana Goid., Rend. R. Acc. Lincei Cl. sc. fis. mat. e. nat. 21: 199-201, 1935.

≡ Ophiostoma catonianum Goid., R. Staz. Pat. Veg. Bol., Rome, n.s. 15: 125-126, 1935.

= Ceratocystis floccosa (Mathiesen) Hunt, Lloydia 19: 36, 1956 fide de Hoog (1974).

≡ Ophiostoma floccosum Mathiesen, Sv. Bot. Tidskr. 45: 219, 1951.

SYNANAMORPHS: (1) Pesotum piceae Crane & Schokneckt, Am. J. Bot. 60: 348-350, 1973. (2) Sporothrix-like to Hyalodendron-like conidial forms.

Colonies attaining a diameter of 44-50 mm in 12 days at 20°C in 2% malt extract agar; slightly floccose becoming floccose to slightly funiculose; colour variable, at first hyaline, then tinged with brownish-yellow, then appearing yellowish-brown mixed with grey, or finally appearing black; often sectored. Pesotum piceae synanamorph: conidiophores macronematous and synnematous; synnemata differentiated into a dark coloured stipe with apical conidiogenous cells borne on penicillate branches. Stipe 165-1,930 µm in length, 11-130 µm wide at the base and 11-78 µm wide

immediately below the conidiogenous apparatus. Conidiogenous cells polyblastic, terminal to intercalary; integrated, sympodial, slightly tapering; hyaline. Conidia holoblastic; hyaline; one-celled; ellipsoidal, ovoid to oblong, often slightly curved, 3.0-6.0 x 1.0-2.0 µm, produced in slimy heads. Sporothrix-like to Hyalodendron-like synanamorph: conidiophores mononematous; macronematous to semi-macronematous. Conidiogenous cells polyblastic, integrated, terminal, determinate, denticulate. Conidia holoblastic; produced either on denticles as sympodulospores in a Sporothrix-like fashion or as acropetalous blastospores in a Hyalodendron-like fashion; hyaline; cylindrical, fusiform to clavate and tapering to their point of attachment; 4.0-10.0 x 1.0-2.0 µm, [(3-)5-15 x 1-3.5 µm, Upadhyay, 1981], often aggregated in slimy heads. Ramoconidia sometimes septate, 6.0-27.5 x 2.0-4.0 µm.

Perithecia developing in culture in two weeks either superficially on the mycelium or partly embedded in the substrate. Bases globose; 100-195 µm in diameter; black; either ornamented with hairs or smooth. Necks straight or curved; black; 530-1,860 µm in length; 22-40 µm wide at the base and 7.5-16.0 µm wide at the tip below the apex. Very long necks often forming annulations due to percurrent proliferations through the previously developed ostioles. Ostiolar hyphae present; hyaline; sometimes septate; straight or divergent; 6.0-25.0 µm long and 1.2-2.5 µm in width at the base. Ascospores hyaline; one-celled; allantoid in side

view, ellipsoid in plan view and spherical in end view, 2.8-4.5 x 1.0-2.2 m; sheath absent; emerging from the ostiole and forming a spore ball at the tip.

NEW ZEALAND HOSTS: Dacrydium cupressinum Lamb., Eucalyptus sp., Larix sp., Pinus radiata D. Don., Podocarpus spicatus R. Br. ex Mirbel, Podocarpus sp., Pseudotsuga menziesii (Mirb.) Franco.

CULTURES ISOLATED[4]: R 133 (a) isolated from Dacrydium cupressinum Minginui Forest; R 133 (b) isolated from Dacrydium cupressinum, Minginui Forest; R 137 (d) isolated from Podocarpus sp., Minginui Forest; 143 (a) isolated from Podocarpus spicatus, Minginui Forest; 143 (b) isolated from Podocarpus spicatus, Minginui Forest; 143 (d) isolated from Podocarpus spicatus, Minginui Forest; 143 (ei) isolated from Podocarpus spicatus, Minginui Forest; 146 (b) isolated from Podocarpus spicatus, Minginui Forest; 146 (c) isolated from Podocarpus spicatus, Minginui Forest; R 147 (a) isolated from Podocarpus spicatus, Minginui Forest; R 147 (b) isolated from Podocarpus spicatus, Minginui Forest; R 147 (c) isolated from Podocarpus spicatus, Minginui Forest; 150 (b) isolated from Pseudotsuga menziesii, Kaingoroa Forest; 154 $(fi)_I$ isolated from Larix sp., Waiotapu Forest; 154 (eiii) isolated from Larix sp., Waiotapu Forest; 163 (fiv) isolated from Pinus radiata, Kaingoroa Forest; R 163 (c) isolated from Pinus radiata, Kaingoroa Forest; R 181 (c) isolated from Eucalyptus sp., Waiotapu Forest.

In his study of wood staining fungi in Germany, Münch (1907) correctly concluded that one of the organisms he encountered,

[4]Only isolates which produced perithecia in vitro are listed.

Plate VI

Ceratocystis piceae (Münch) Bakshi

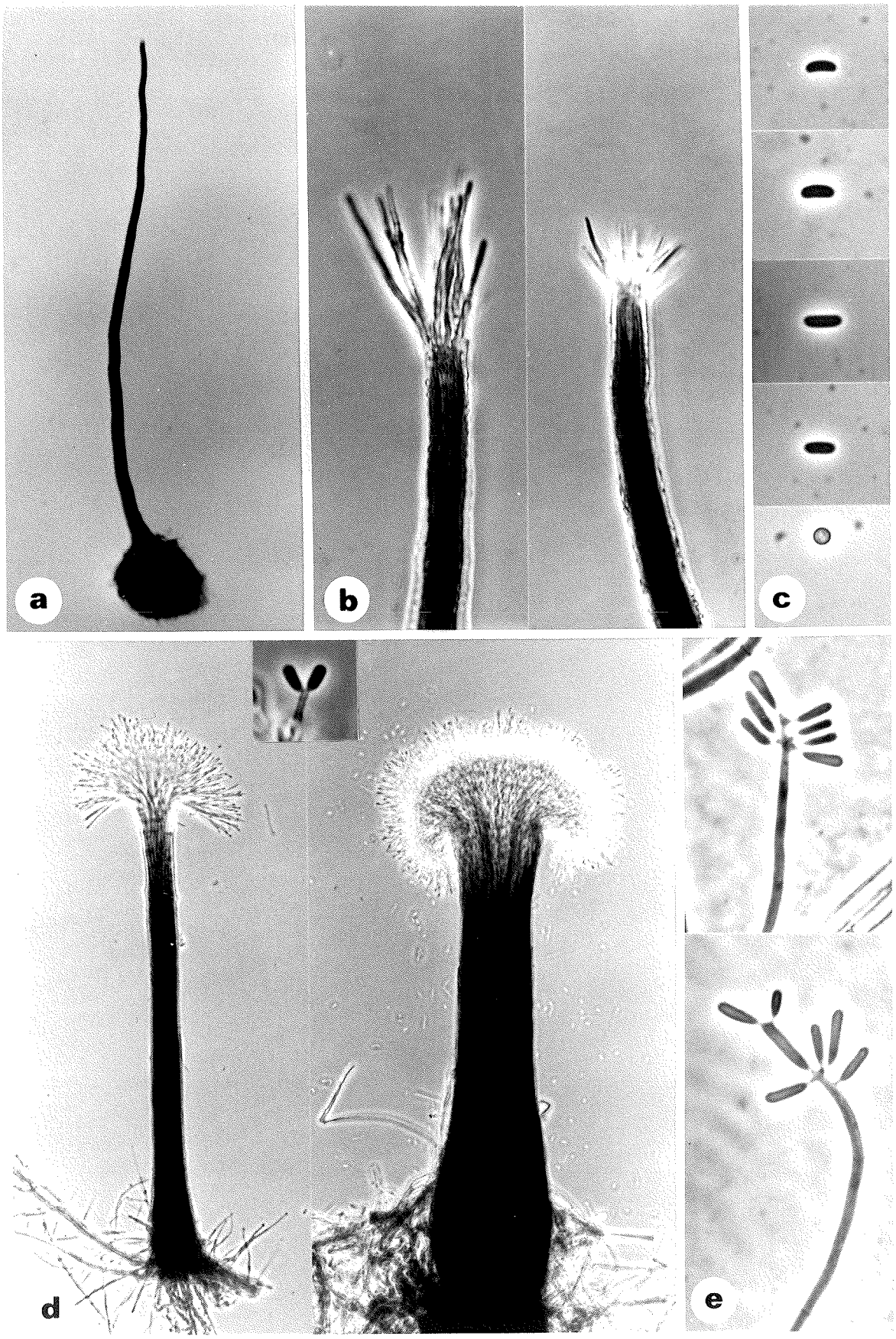
Fig. a. Perithecium (X95).

Fig. b. Ostiolar hyphae (X615).

Fig. c. Ascospores in side, plan and end views (X1500).

Fig. d. Synnemata of *Pesotum* state (X245), inset shows sympodulospores on conidiogenous cell (X1500).

Fig. e. Mononematous anamorphic state. *Sporothrix*-like in upper photograph and *Hyalodendron*-like in lower photograph (X1500).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Ceratostomella pilifera (Fr.) Wint., was in fact a composite species comprised of several distinct entities. One of these entities he described as Ceratostomella piceae whose imperfect state, he noted, belonged in the genus Graphium of the Fungi Imperfecti and "warscheinlich mit Graphium penicillioides Corda identisch ist." Many subsequent workers have followed his lead; they too considering the anamorph of Ceratocystis piceae (Münch) Bakshi (1950) to represent G. penicillioides. However, Crane and Schoknecht (1973) studying Ceratocystis ulmi, C. piceae and G. penicillioides concluded that the anamorphic states of C. ulmi and C. piceae represented a new, undescribed, anamorphic genus, from which G. penicillioides was totally distinct. The new anamorph genus proposed was Pesotum, and its species were characterized by conidia produced as sympodulospores, while G. penicillioides produced its conidia as annellospores.

Being cosmopolitan in distribution, C. piceae was one of the most commonly encountered organisms during this study. It has previously been recorded from New Zealand by Butcher (1968) who had isolated it on several occasions from red beech [Nothofagus fusca (Hook.) Oerst.] from the west coast of the South Island; he also reports that C. piceae had been isolated from silver beech [Nothofagus menziesii (Hook.) Oerst.] in New Zealand.

Of the long list of mycologists who have worked with C. piceae, all have found that this organism loses its ability to produce perithecia in culture quite rapidly. In addition, the Pesotum

state is reported as often being lost over time, leaving only the Sporothrix-like to Hyalodendron-like state. In this study, many isolates were obtained in culture which resembled C. piceae closely except no perithecia were produced even when subjected to a large range of different growing conditions. The possibility that failure to obtain perithecia with such isolates was related to a sexual mechanism was considered, particularly since the question as to whether this organism is homothallic or heterothallic has bothered mycologists since the days of Münch. All published reports indicate that it is homothallic [e.g. Mittmann 1932 with Ceratostomella quercus = C. piceae fide Hunt (1956); Goidanich 1935 with Ophiostoma catonianum = C. piceae fide de Hoog (1974); Bakshi 1951a]. However, Münch (1907) and MacCallum (1922) found that starting cultures from the conidia of the Pesotum state yielded only the Pesotum and Sporothrix-like to Hyalodendron-like states; MacCallum added that starting cultures from ascospores always resulted not only in the Sporothrix-like to Hyalodendron-like state and Pesotum state but, finally the perithecia of C. piceae. He stated Münch had exactly similar results (true) and he (MacCallum) stated that this led Münch to acknowledge such results were very much against the theory that the Graphium was a stage in the life history of C. piceae. The present study has also found that cultures started from ascospore ooze resulted in production of the two conidial states followed by production of perithecia, whereas cultures started from the conidia of the

Pesotum state, either as masses of conidia or as single spores, never produced perithecia, although both conidial forms were present.

To investigate whether C. piceae is heterothallic, different isolates of what were believed to represent the anamorphic states of C. piceae were crossed in as many different combinations as possible. In only a single cross did perithecia form. However, no conclusion can be drawn since an isolate started from a single conidium from the Pesotum state was found which would produce perithecia in culture if grown at a temperature of 9°C for several months. Subsequently it was also found that two isolates started from conidia of the Pesotum state produced perithecia on wood when the wood sample has been accidentally contaminated with a species of Aspergillus Micheli ex Fries; the Aspergillus may be producing a compound needed by C. piceae for perithecial production. It is quite possible therefore that C. piceae possesses strains which can be either homothallic or heterothallic. Mathiesen (1950b) found amongst the cultures of C. piceae which she studied, an isolate from beetle-infested wood which produced perithecia in culture, while a second isolate from wood not infested by beetles produced the imperfect states only. She implies that both mating types were present in the beetle-infested wood while only one mating type was present in the non-infested wood; bark beetles are known vectors of Ceratocystis spp.

6. Ceratocystis piceaperda (Rumb.) C. Moreau, Rev. Mycol. Suppl. Colonial, 17: 22, 1952 Plate VII, Figs. a-e.

≡ Ceratostomella piceaperda Rumb., J. Agric. Res. 52: 432, 1936.

≡ Grosmannia piceaperda (Rumb.) Goid., R. Staz. Pat. Veg. Bol., Rome, n.s. 16: 255, 1936.

≡ Ophiostoma piceaperdum (Rumb.) Arx, Antonie van Leeuwenhoek 18: 211, 1952.

= Ceratocystis europhioides Wright & Cain, Can. J. Bot. 39: 1222, 1961 fide Upadhyay (1981).

ANAMORPH: Leptographium sp.

Colonies attaining a diameter of 90 mm in 7 days at 20°C on 2% malt agar. Colony appressed; at first hyaline, then turning dark brown in the centre and finally the entire colony turning black. Conidiophores mononematous and macronematous; differentiated into a dark-coloured stipe with conidiogenous cells borne on penicillate branches; stipe 150-290 µm in length and 6.0-10.0 µm in width. Conidiogenous cells terminal, integrated; hyaline; variable. Appearing (1) holoblastic, percurrent, annellate; (2) polyblastic and sympodial; or rarely (3) enteroblastic, percurrent, and phialidic(?). Conidia hyaline; one-celled; oblong to clavate to obovoid; rounded at their apices, but truncate at their point of attachment, usually with a definite frill; 4.0-8.5 x 1.5-4.0 µm, produced in slimy heads.

In culture, perithecia developing either superficially on the

colony or immersed (often deeply) in the agar, within 30 days; black; bases globose; 160-335 µm in diameter. Necks black; curved, straight or tortuous; 260-1000 µm in length, 40-70 µm wide at the base and 22-54 µm wide at the tip; ostiolar hyphae absent. Ascospores hyaline; one-celled, appearing reniform in side view, oblong in plan view and spherical in end view; covered by a hyaline sheath; appearing cucullate in side view, quadrangular in plan view and triradiate in end view; 4.0-5.5 x 3.0-4.8 µm including sheath [(4-)4.6-7.2(-8) x 2-3.5(-4) µm, Upadhyay 1981]; emerging from the ostiole as spiral forming cirrhi.

NEW ZEALAND HOSTS: Eucalyptus sp., Pinus nigra Arnold, Pinus radiata D. Don, Pinus taeda L.

CULTURES ISOLATED: R 14 (b) isolated from Pinus nigra, Kaingoroa Forest; R 43 isolated from Pinus radiata, Woodhill Forest; R 63 isolated from Pinus radiata, Tairua Forest; R 65 (a) isolated from Pinus radiata, Tairua Forest; R 76 (a) isolated from Pinus radiata, Tairua Forest; R 76 (b) isolated from Pinus radiata, Tairua Forest; R 116 (a) isolated from Pinus radiata, Woodhill Forest; 163 (bi) isolated from Pinus radiata, Kaingoroa Forest; 163 (c) isolated from Pinus radiata, Kaingoroa Forest; 163 (e) isolated from Pinus radiata, Kaingoroa Forest; R 163 (a) isolated from Pinus radiata, Kaingoroa Forest; R 163 (d) isolated from Pinus radiata, Kaingoroa Forest; R 173 isolated from Pinus nigra, Kaingoroa Forest; R 174 (a) isolated from Pinus nigra, Kaingoroa Forest; R 174 (b)' isolated from Pinus nigra, Kaingoroa Forest; R 174 (c) isolated from

Plate VII

Ceratocystis piceaperda (Rumbold) C. Moreau

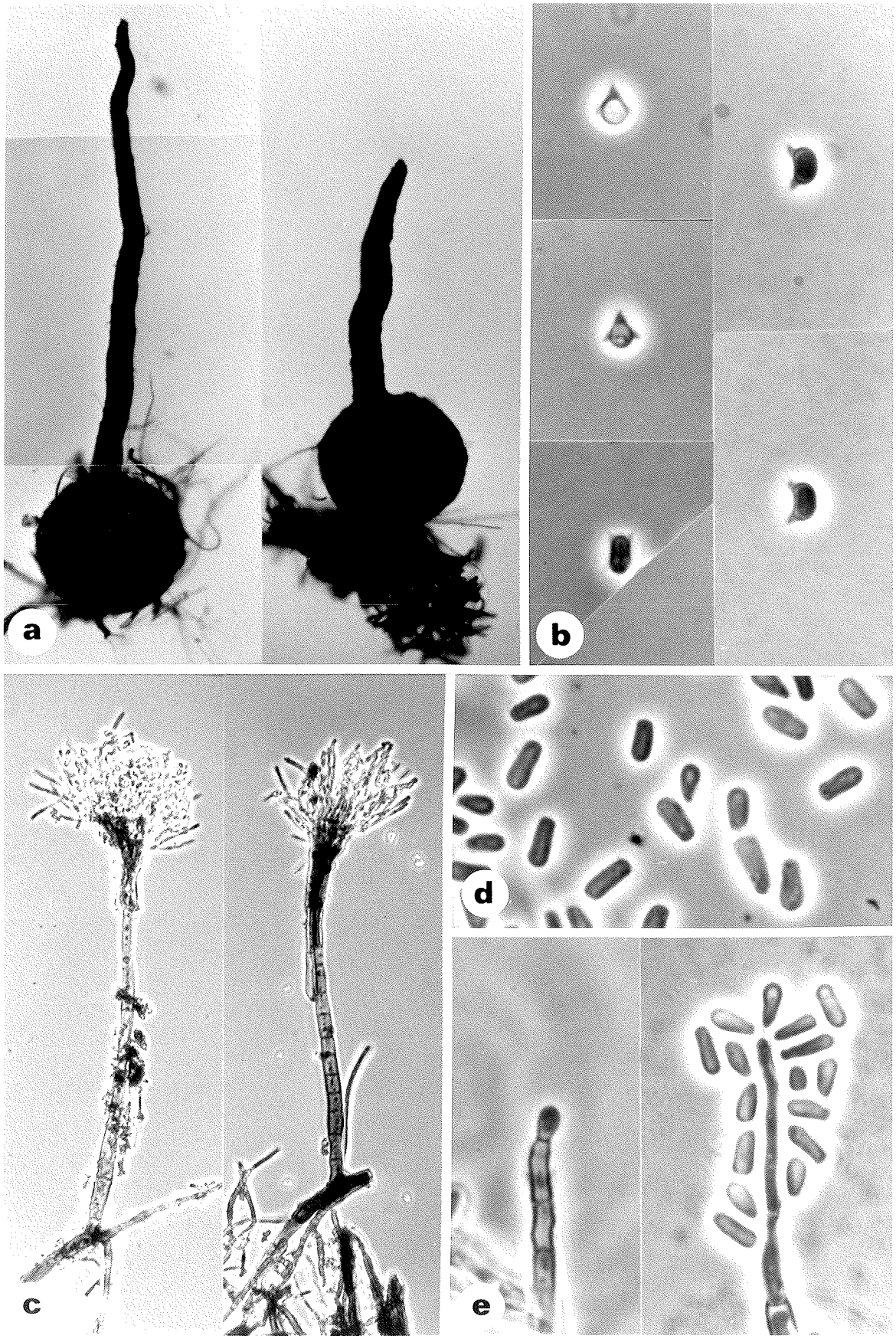
Fig. a. Perithecia (X90).

Fig. b. Ascospores; end, plan and side views (X1750).

Fig. c. *Leptographium* anamorph (X280).

Fig. d. Conidia (X1750).

Fig. e. Conidiogenous cells (X1750).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Pinus nigra, Kaingoroa Forest; R 174 (e) isolated from Pinus nigra, Kaingoroa Forest; R 183 (a) isolated from Eucalyptus sp., Riverhead Forest; R 183 (a)' isolated from Eucalyptus sp., Riverhead Forest; R 183 (b) isolated from Eucalyptus sp., Riverhead Forest; R 183 (b)' isolated from Eucalyptus sp., Riverhead Forest; R 183 (c)' isolated from Eucalyptus sp., Riverhead Forest; R 183 (d) isolated from Eucalyptus sp., Riverhead Forest; R 183 (e) isolated from Eucalyptus sp., Riverhead Forest; R 183 (f) isolated from Eucalyptus sp., Riverhead Forest; R 185 (a) isolated from Pinus taeda, Riverhead Forest; R 185 (b) isolated from Pinus taeda, Riverhead Forest; R 185 (c) isolated from Pinus taeda, Riverhead Forest; R 185 (d) isolated from Pinus taeda, Riverhead Forest; R 194 (d) isolated from Pinus nigra, Riverhead Forest; R 200 (a) isolated from Pinus radiata, Riverhead Forest; R 201 (a) isolated from Pinus radiata, Riverhead Forest; R 201 (b) isolated from Pinus radiata, Riverhead Forest; R 201 (c) isolated from Pinus radiata, Riverhead Forest.

Upadhyay (1981) reduced C. europhioides to synonymy with C. piceaperda without explaining why he did so; however if one compares written descriptions of the two organisms they appear almost identical. With this in mind, few differences exist between the New Zealand isolates of C. piceaperda and published descriptions of isolates found in North America (Rumbold 1936; Wright and Cain 1961; Davidson et al. 1967).

The Leptographium anamorphic state is cause for confusion as it appears to have the ability to form three types of conidiogenous

cells. This ability is not unusual as many members of the Graphium and Leptographium complexes can do this, but it does present difficulties in identification. For now, Leptographium appears to be the safest genus for this anamorph as opposed to Verticicladiella or Phialocephala.

7. Ceratocystis pilifera (Fr.) C. Moreau, Rev. Mycol. Suppl. Colonial 17: 22, 1952 Plate VIII, Figs. a-e.

≡ Sphaeria pilifera Fr., Syst. Mycol. 2: 472, 1822.

≡ Ceratostoma piliferum (Fr.) Fuckel, Symb. Mycol., 128, 1870.

≡ Ceratostomella pilifera (Fr.) Winter, Rabenh. Kryptogamen-Flora, 1: 252, 1887.

≡ Linostoma piliferum (Fr.) Höhn, Annls. mycol. 16: 91, 1918.

≡ Ophiostoma piliferum (Fr.) H. & P. Sydow, Annls. mycol. 17: 43, 1919.

= Ceratocystis coerulea (Münch) C. Moreau, Rev. Mycol. Suppl. Colonial 17: 22, 1952 fide Hunt (1956).

≡ Ceratostomella coerulea Münch, Naturw. Ztschr. f. Forst. u. Landw. 5: 561, 1907.

≡ Ophiostoma coeruleum (Münch) H. & P. Sydow, Annls. mycol. 17: 43, 1919.

= Ceratostomella echinella Ell & Ev. emend. Hedgc., Mo. Bot. Gard. Ann. Rpt. 17: 69, 1906 fide Hunt (1956).

≡ Ophiostoma echinellum (Ell & Ev. emend. Hedgc.) H. & P. Sydow, Annls. mycol. 17: 43, 1919.

= Ceratocystis ambrosia Bakshi, Trans. Br. mycol. Soc. 33: 116, 1950 fide Griffin (1968).

= Ceratocystis longirostellata Bakshi, Mycol. Pap. 35: 8, 1951 fide Upadhyay (1981).

≡ Ophiostoma longirostellatum (Bakshi) von Arx & Miller, Beitr. Krypt. Fl. Schweiz II-I: 395, 1954.

= Ceratocystis capillifera (Hedgc.) C. Moreau, Rev. Mycol. Suppl. Colonial 17: 22, 1952 fide Griffin (1968).

≡ Ceratostomella capillifera Hedgc., Mo. Bot. Gard. Ann. Rept. 17: 71, 1906.

≡ Ophiostoma capilliferum (Hedgc.) H. & P. Sydow, Ann. Mycol. 17: 43, 1919.

= Ceratocystis schrenkiana (Hedgc.) C. Moreau, Rev. Mycol. Suppl. Colonial 17: 22, 1952 fide Griffin (1968).

≡ Ceratostomella schrenkiana Hedgc., Mo. Bot. Gard. Ann. Rept. 17: 67, 1906.

≡ Ophiostoma schrenkianum (Hedgc.) H. & P. Sydow, Annls. mycol. 17: 43, 1919.

ANAMORPH: Sporothrix-like to Hyalodendron-like.

Colonies attaining a diameter of 66-70 mm in 12 days at 20°C on 2% malt extract agar. Colony flocculose to floccose, to somewhat funiculose; at first white and then becoming dark brown or remaining white with dark brown sectors. Conidiophores mononematous, micronematous to semi-macronematous; hyaline. Conidiogenous cells poly-

blastic; terminal to intercalary; sympodial. Conidia holoblastic; produced either on denticles as sympodulospores in a Sporothrix-like fashion or as acropetalous blastospores in a Hyalodendron-like fashion; hyaline; one-celled, cylindrical, fusiform to clavate and tapering to their point of attachment; 4.5-10.0 x 1.0-2.0 µm. Ramoconidia 8.0-27.0 x 1.5-2.8 µm.

Perithecia developing in culture superficially on the substrate within two weeks. Bases globose; 145-275 µm in diameter; black and ornamented with hairs. Necks straight or curved; black in colour; 1.2-2.4 mm in length, 27-50 µm wide at base, 12-19 µm wide at the tip immediately below the apex; annulations frequently forming along the necks due to percurrent proliferations through the neck apex. Ostiolar hyphae present; hyaline, septate, divergent; 17-75 µm long and 0.8-1.8 µm wide at the base. Ascospores hyaline; one-celled; allantoid to orange-section shaped in side view, oval in plan view and spherical in end view; 4.0-5.0 x 1.0-1.5(-2) µm; sheath absent; emerging from the ostiole and forming spore ball at tip.

NEW ZEALAND HOST: Pinus radiata D. Don

CULTURES ISOLATED: R 129 (b) isolated from Pinus radiata, Woodhill Forest; R 129 (d) isolated from Pinus radiata, Woodhill Forest.

C. pilifera is the organism to which blue staining of wood was first attributed by Robert Hartig (1878), the famous 19th century German forester. C. pilifera is reported in the pioneer studies on wood staining by fungi (e.g. von Schrenk 1903; Hedgcock

Plate VIII

Ceratocystis pilifera (Fr.) C. Moreau

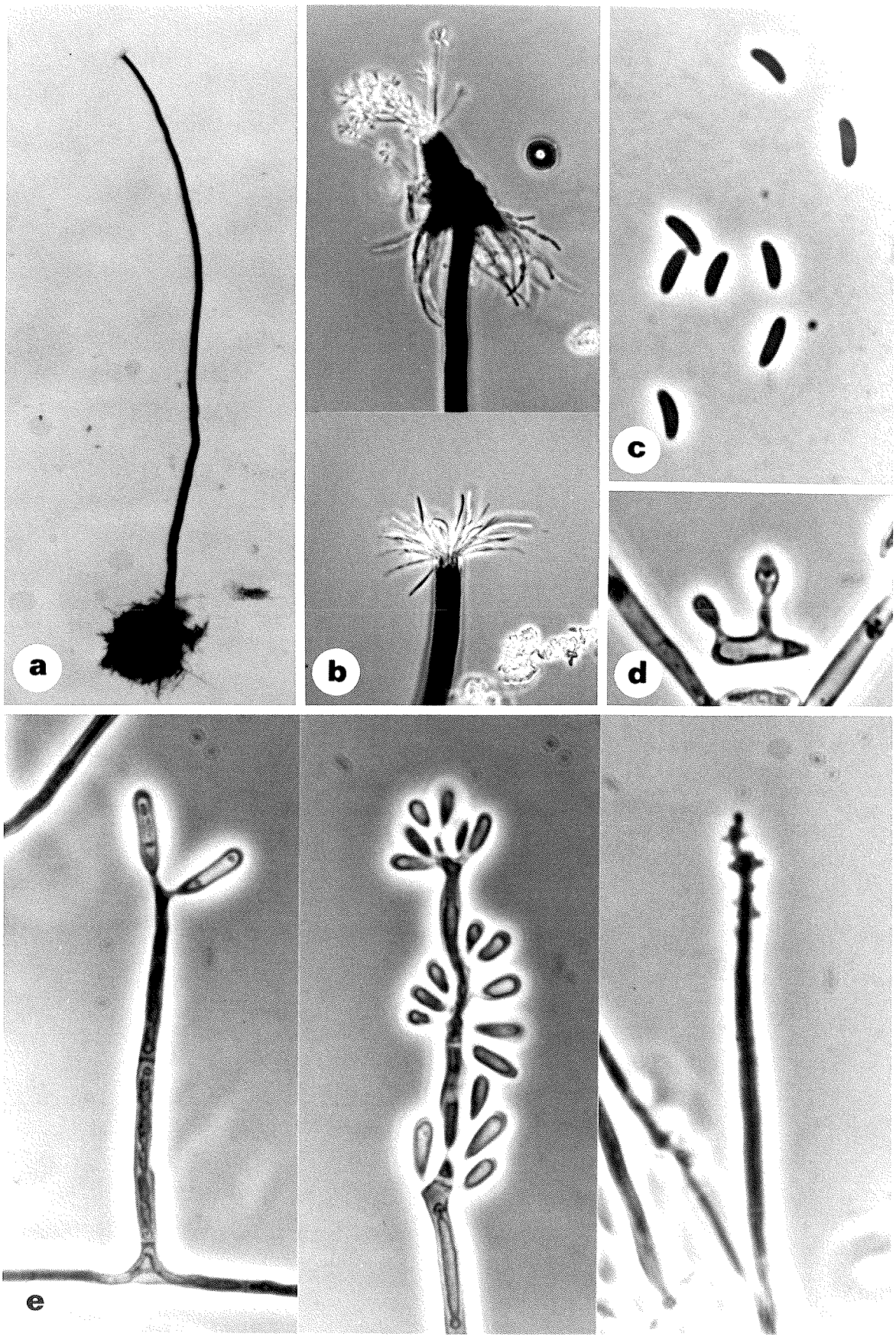
Fig. a. Perithecium (X50).

Fig. b. Neck apices showing proliferation through the ostiole with conidial state in upper photograph, and ostiolar hyphae at apex in lower photograph (X300).

Fig. c. Ascospores (X1900).

Fig. d. Ramoconidium with secondary conidia (X1900).

Fig. e. *Sporothrix*-like anamorphic state (X1900).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1906; Münch 1907) and since those earliest records has been reported very frequently in studies throughout the world. There have been two previous reports of C. pilifera from Australasia, both of which involved isolations made from Pinus radiata; Eckersley (1934) in the state of Victoria in Australia and Yeates (1924) in New Zealand. While no name is given to the organism described by Yeates, the description and illustrations clearly represent C. pilifera.

At times the anamorphic state appears to represent a Sporothrix sp. while at other times it can be similar to a Hyalodendron sp. This variability has been noted in the present isolates as it has in all previous studies of this organism since the days of Hedgcock (1906).

In culture, C. pilifera produces what appear to be protoperithecia. This has been recorded in the literature by various workers [e.g. Eckersley 1934; Bakshi 1950 for C. ambrosia (= C. pilifera fide Griffin, 1968); Griffin 1968] who have called these structures sclerotia. However Mittmann (1932) in her cultural studies of certain species of Ceratocystis found that C. coerulea (= C. pilifera fide Hunt, 1956) was heterothallic and that these sterile protoperithecia which later authors called sclerotia were formed in cultures derived from a single mating type strain and, when opposite mating types were combined, normal perithecia developed. Although crossing experiments were not undertaken in this study, the protoperithecia formed in cultures eventually developed into mature perithecia thus indicating the presence of both mating types

in the isolates.

8. Ceratocystis rostrocoronata Davids. & Eslyn, Mem. New York Bot. Gard., 28: 50-51, 1976 Plate IX, Figs. a-e.

ANAMORPH: Sporothrix sp.

Colonies attaining a diameter of 30 mm in 12 days at 20°C on 2% malt agar; appressed, becoming flocculose to slightly funiculose; at first hyaline, centre becoming reddish brown and finally becoming dark-grey, with edges remaining hyaline. Conidiophores mononematous to semi-macronematous. Conidiogenous cells polyblastic, integrated, terminal, determinate; denticulate. Conidia produced sympodially upon the denticles; hyaline; one-celled; ovoid, obovoid, fusiform; tapering towards the base; sometimes curved; apex obtuse to almost truncate; when truncate, giving the conidia a triangular appearance and having "ear-like" projections; 2.0-5.0 x 0.8-2.0 µm.

Perithecia developing superficially on both the mycelium in culture and on the natural substrate. Bases globose, subglobose to broadly ellipsoidal; black, and ornamental with dark-brown hyphal elements; 70-140 µm in diameter. Perithecial necks black; straight or curved, 230-600 µm long, 15-33 µm wide at the base, and 7.5-10.0 µm wide at the tip (just below the apex). Ostiolar hyphae present at the neck apex; hyaline, divergent 6.0-12.5 µm long and 1.0 µm wide at the base. Ascospores hyaline, one-celled;

allantoid to orange-section shaped in side view, oval to fusiform in plan view, globose to spherical in end view; 3.5-5.0 x 1.0-1.5 µm; sheath absent, emerging from ostiole and being contained within a clear liquid drop.

NEW ZEALAND HOST: Eucalyptus sp.

CULTURES ISOLATED: R 197 (a) isolated from Eucalyptus sp., Riverhead Forest; R 197 (b) isolated from Eucalyptus sp., Riverhead Forest; R 197 (c) isolated from Eucalyptus sp., Riverhead Forest; R 197 (d) isolated from Eucalyptus sp., Riverhead Forest; R 197 (e) isolated from Eucalyptus sp., Riverhead Forest; R 197 (f) isolated from Eucalyptus sp., Riverhead Forest.

When C. rostrocoronata was described by Eslyn and Davidson (1976) its distinguishing characteristic was the crown of a very short ostiolar hyphae at the tip of the neck (Fig.c, Plate IX) where small droplets of ascospores developed (unlike most Ceratocystis spp. which develop large droplets). This characteristic was observed in the New Zealand isolates; however the ostiolar hyphae were entirely hyaline rather than possessing any light brown pigmentation. Perithecia developed slowly and sparsely in agar culture but they developed very quickly (within 30 days) and very abundantly when grown on autoclaved pine wood discs.

The real differences between the New Zealand isolates and those described by Eslyn and Davidson, and Upadhyay (1981) lie within the Sporothrix anamorph. The conidia of the Sporothrix

Plate IX

Ceratocystis rostrocoronata Eslyn & Davidson

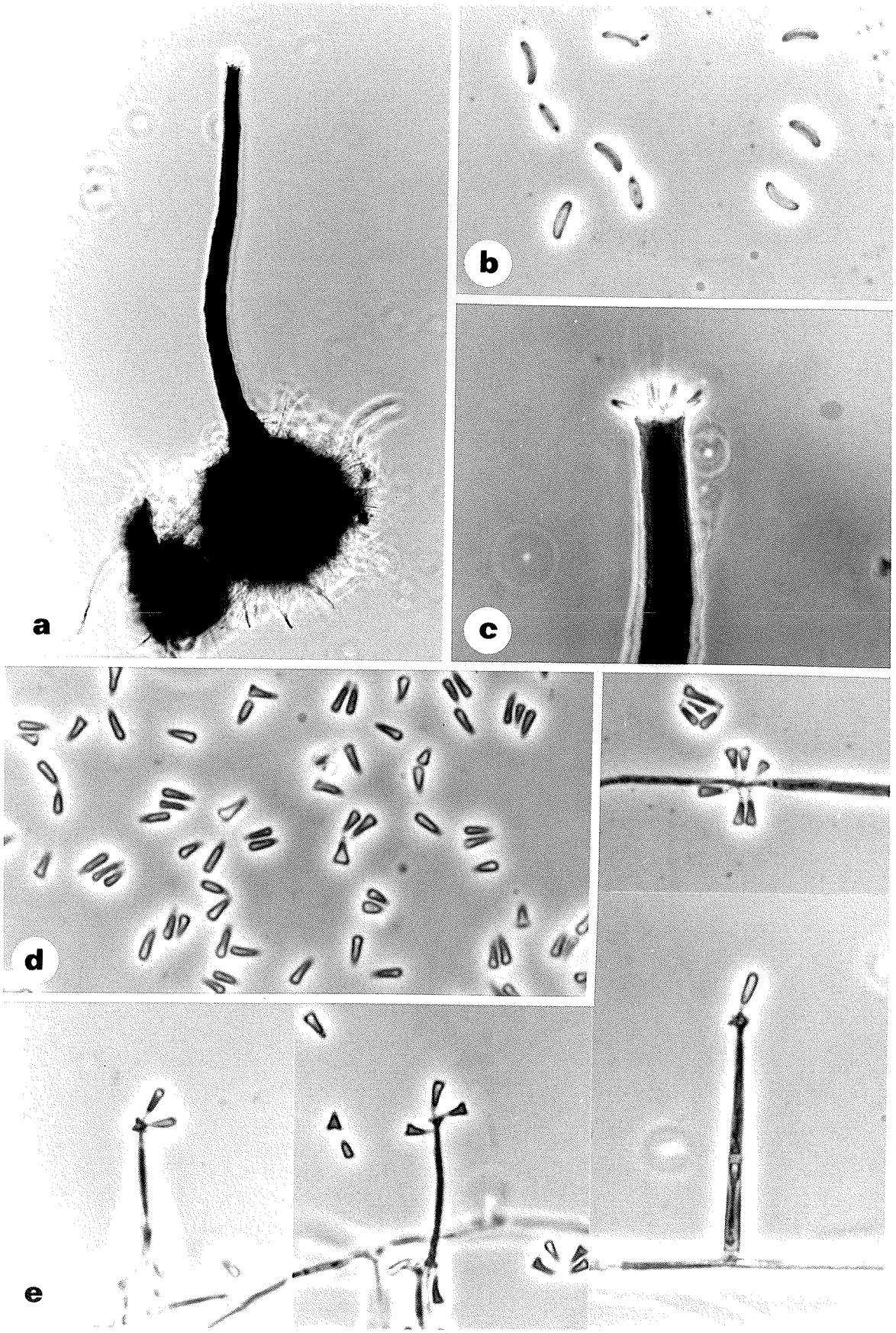
Fig. a. Perithecium (X260).

Fig. b. Ascospores (X1650).

Fig. c. Perithecial neck apex showing ostiolar hyphae (X850).

Fig. d. Conidia (X1650).

Fig. e. *Sporothrix* anamorph (X1650).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anamorph from both wood and agar culture of the New Zealand isolates were extremely variable in shape including triangular shaped conidia which were not found by either Eslyn and Davidson or Upadhyay. Triangular shaped conidia have been found under certain conditions in the Sporothrix state of Ceratocystis stenoceras (Robak) C. Moreau and in Sporothrix schenkii (in Mariat and Diez, 1971) and in the Sporothrix state of Ceratocystis deltoideospora Olchow. & Reid (1974).

9. Sphaeronaemella fimicola Marchal, Bull. Soc. Roy. Bot. Belg. 30: 143, 1891 Plate X, Figs. a-e.

≡ Sphaeronema fimicola (Marchal) Jacz., Nouv. Mem. Soc. Natur. de Moscou, n.s. 15: 300, 1898.

≡ Ceratocystis fimicola (Marchal) Upadhyay, Monogr. Ceratocystis and Ceratocystiopsis, p.95, 1981.

≡ Viennotidia fimicola (Marchal) Cannon & Hawksw. (as Viennotidea fimicola) J. Linn. Soc., Bot. 84: 157, 1982.

= Sphaeronaemella carnea Ellis & Everh., J. Mycol. 6: 152, 1890 fide Pease (1948).

ANAMORPH: Gabarnaudia fimicola Samson & Gams, Stud. Mycol. 6: 92, 1974.

Colonies attaining a diameter of 60-64 mm in 12 days at 20°C on 2% malt agar. Colony floccose; white in colour. Conidiophores mononematous, micronematous to very rarely semi-macronematous;

hyaline and irregularly branched; 70-150 µm (up to 200 µm according to Samson 1974). Conidiogenous cells enteroblastic, integrated, terminal, discrete and determinate; variable in length but up to 85 µm long by 2.5-3.5 µm wide at the base and 1.8-2.5 µm at their tips; cylindrical to lanceolate. Phialospores extruded in long chains; hyaline; oval to fusiform with a truncate base; 11.0-23.0 x 2.0-4.0 µm.

Perithecia developing superficially on the mycelium within two weeks after inoculation; pale-yellow to orange in colour. Bases globose to subglobose, 215-380 µm in diameter (120-300 µm according to Cannon and Hawksworth, 1982), pale-yellow to orange; smooth. Necks straight or slightly curved, pale-yellow to orange, 1.4-2.2 mm long (500-900 µm, Cannon and Hawksworth); 45-70 µm wide at the base, 17-32 µm wide at the tip immediately below the ostiolar hyphae. Ostiolar hyphae hyaline, straight to divergent; 68-175 µm long and 2.5-5.0 µm wide at the base. Ascospores hyaline; one-celled; allantoid to orange-section shaped in side view; oblong with obtuse ends in plan view and spherical in end view, 6.0-8.0 x 1.75-2.5 µm; sheath absent; emerging from the ostiole and forming a spore ball at the tip.

NEW ZEALAND HOST: Pinus radiata D. Don.

CULTURES ISOLATED: R 55 (a) isolated from Pinus radiata, Tairua Forest.

Although S. fimicola has been isolated almost exclusively from the dung of herbivores and carnivores by various researchers such

Plate X

Sphaeronaemella fimicola Marchal

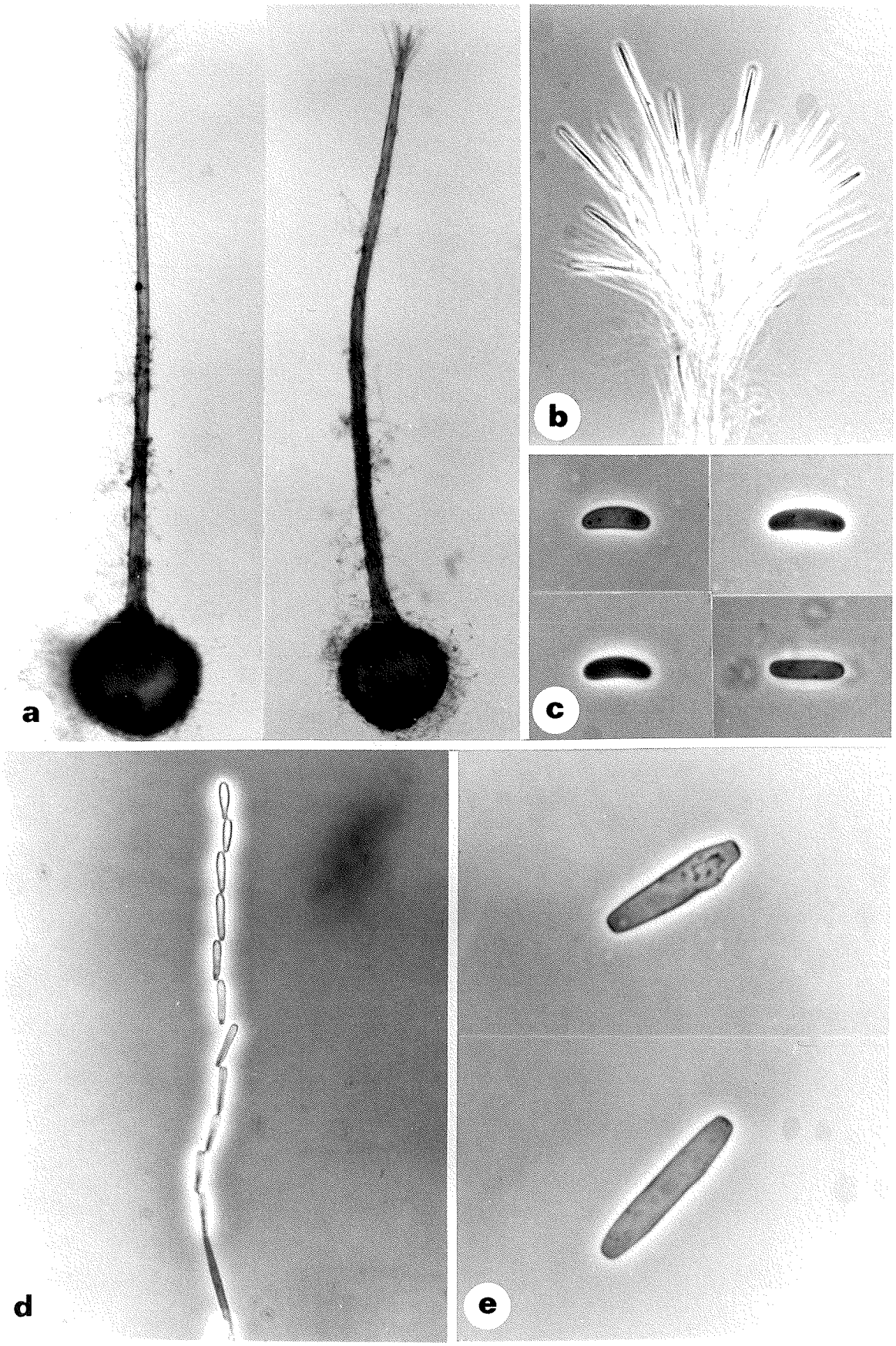
Fig. a. Perithecia (X100).

Fig. b. Ostiolar hyphae (X220).

Fig. c. Ascospores in side and plan views (X1650).

Fig. d. Phialide of Gabarnaudia state with chain of conidia (X670).

Fig. e. Conidia (X1650).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as Cain and Weresub (1957), during this study an isolate was obtained from wood. Other exceptions to the normally reported substrates for this species are by Ellis and Everhart (1890) who isolated and described Sphaeronaemella carnea from the bark of ash and Pease (1948) who, in her study of S. fimicola, isolated it from rotting curcurbits, and considered S. carnea to be synonymous with S. fimicola.

Various workers who have studied this organism in detail (e.g. Pease 1948; Cain and Weresub 1957; Samson 1974) have found that S. fimicola loses its ability to produce perithecia in vitro very quickly and only by inoculation with very large masses of ascospores will perithecial formation occur in culture; this was also true for the isolate from New Zealand. Cain and Weresub (1957) noted that perithecial formation was stimulated when S. fimicola was grown in mixed culture with Aspergillus repens (Corda) Sacc. Samson (1974) could not duplicate Cain and Weresub's results, although by using bacteria and other dung fungi (e.g. Sporormia sp.) he was able to induce ascocarp development.

Upadhyay (1981) reduced the genus Sphaeronaemella, including S. fimicola, to synonymy with Ceratocystis. He felt that the presence or absence of pigmentation in the ascocarps was not important as a generic criterion. While pigmentation may not be important, because no organism normally assigned to the genus Ceratocystis has an anamorphic state referrable to Gabarnaudia, and because Sphaeronaemella species are found on substrates

unlike those of typical Ceratocystis spp. (e.g. dung for S. fimicola and members of the Helvellaceae for S. helvellae as compared to herbaceous or woody plants for Ceratocystis spp.), Sphaeronaemella should be retained as a discrete genus.

Cannon and Hawksworth (1982) revised the taxonomy of Sphaeronaemella based on examination of morphological features employing scanning electron microscopy. They felt that Sphaeronaemella should be monotypic and contain only S. helvellae, the original type species. In their study they found that S. helvellae possesses what they interpret as narrow longitudinal germ-slits in the ascospores; these could only be visualized with the aid of SEM. The remaining species of Sphaeronaemella including S. fimicola were transferred to the genus Viennotidia because of: (1) the occurrence of a subapical germ pore near one end of the ascospore rather than germ slits (again visible only by electron microscopy); (2) the presence of a Gabarnaudia anamorph (no definite anamorph has been connected with S. helvellae according to Cannon and Hawksworth, 1982); and (3) S. helvellae is obligately fungicolous while those species placed in the genus Viennotidia are dung, soil, and plant saprophytic fungi. The illustration used by Cannon and Hawksworth show these germ pores as being simple depressions on the ascospores; these have not been seen during this study using light microscopy. It is a well known fact that preparation of fungal specimens for SEM often results in collapsed or shrunken structures which when observed can be easily misinterpreted. Therefore TEM should be

used to observe the alleged pores to see whether in fact they do exist. Employing SEM as a means of identifying fungi defeats the purpose of an easily workable taxonomic system. If separating genera on the basis of possessing a germ slit as opposed to a germ pore is used, and if these structures can only be seen by using a SEM, this will create problems for people without an SEM. Thus while germ slits and pores have merit in taxonomy, only structures observable under the light microscope should be taken into account. S. fimicola should not be transferred to the genus Viennotidia, but remain in the genus Sphaeronaemella. In fact, Malloch (1974) correctly reduced Viennotidia to synonymy with Sphaeronaemella.

When Viennotidia was erected by Negru and Verona (1966) they failed to designate a type species and thus the genus was invalidly published. Cannon and Hawksworth (1982) therefore took up the name Viennotidea, but designated Viennotidea fimicola (Marchal) Cannon & Hawks. comb. nov. as the holotype and, since Negru and Verona (1966) were unfamiliar with V. fimicola, Cannon and Hawksworth claimed Viennotidea as a newly validated genus attributable to themselves, i.e. Viennotidea P. Cannon & D. Hawksw. gen. nov. (1982), (a misspelling). However they were obviously unaware that Rogerson (1970) validated the genus when he selected V. spermosphaerici Negru & Verona as the lectotype of the genus and therefore Viennotidea stands as follows (correctly spelled): Viennotidia Negru & Verona ex Rogerson (1970) not 1971 as listed in the Dictionary of the Fungi and the valid date of publication of, and

citation of the type, is as follows: Viennotidia spermosphaerici Negru & Verona ex Rogerson (1970). Viennotidia Cannon & Hawksworth (1982) (as Viennotidea) becomes a later homonym of Viennotidia Negru & Verona ex Rogerson as it is based on a different nomenclatural type. However, the correct citations for the other members of Viennotidia are as follows: Viennotidia fimicola (Marchal) P. Cannon & D. Hawksw. (1982) (as Viennotidea fimicola), Viennotidia humicola (Samson & Gams) P. Cannon & D. Hawksw. (1982) (as Viennotidea humicola) and Viennotidia raphani (Malloch) P. Cannon & D. Hawksw. (1982) (as Viennotidea raphani).

(b) Wood Staining Pyrenomycetes

10. Coniochaeta velutina (Fuckel) Munk, Dansk Bot. Ark. 12: 11, 1948 Plate XI, Figs. a-d.

Rosellinia velutina Fuckel, Symb. mycol. p.149, 1870.

ANAMORPH: Phialophora luteo-viridis (Beyma) Schol-Schwarz Persoonia 6: 79, 1970.

Colonies attaining a diameter of 20-26 mm in 12 days at 20°C on 2% malt agar. Colony appressed to floccose; at first pale-yellow, then centre becoming very dark greyish-brown, then very dark-brown and finally reddish-brown, while the margin of the colony remains reddish-yellow. Conidiophores micronematous to occasionally semi-macronematous; straight to sparingly branched;

pale-brown. Conidiogenous cells monophialidic, very rarely polyphialidic; integrated and terminal or discrete; ampulliform to cylindrical, often with a prominent collarette. Conidia aggregated in slimy heads; one-celled; hyaline; ovoid to turbinate; 2.0-4.0 x 1.0-2.5 µm.

Perithecia gregarious; developing superficially or slightly embedded in the substrate. Bases globose to broadly ellipsoidal; black; 185-240 µm in diameter and ornamented with perithecial hairs 20-60 µm long, 2.5-3.75 µm wide. Necks black, conical to almost non-existent. Asci cylindrical, but tapering at their bases; 53-78 x 5.0-7.5 µm and containing eight uniseriate ascospores. Ascospores dark-brown, one-celled, ellipsoidal to broadly ellipsoidal; 5.5-8.0 x 4.0-5.5 µm (7.0-10.0 x 4.0-7.0 µm, Taylor, 1970).

NEW ZEALAND HOST: Pinus radiata D. Don.

CULTURE ISOLATED: 94 (b) isolated from Pinus radiata, Whangapoua Forest.

C. velutina is an organism which has been found on many different substrates such as wood, dung, and soil (Mahoney and La Favre, 1981). Basham et al. (1969) studying the ecology of C. velutina on Acer saccharum Marsh, isolated the fungus from stained and decayed wood; the organism always being found associated with trunk wounds and with other fungi. Mahoney and La Favre report that Coniochaeta spp. are of low virulence on most hosts, usually appearing on dead tissue or as opportunistic invaders of previously

Plate XI

Coniochaeta velutina (Fuckel) Munk

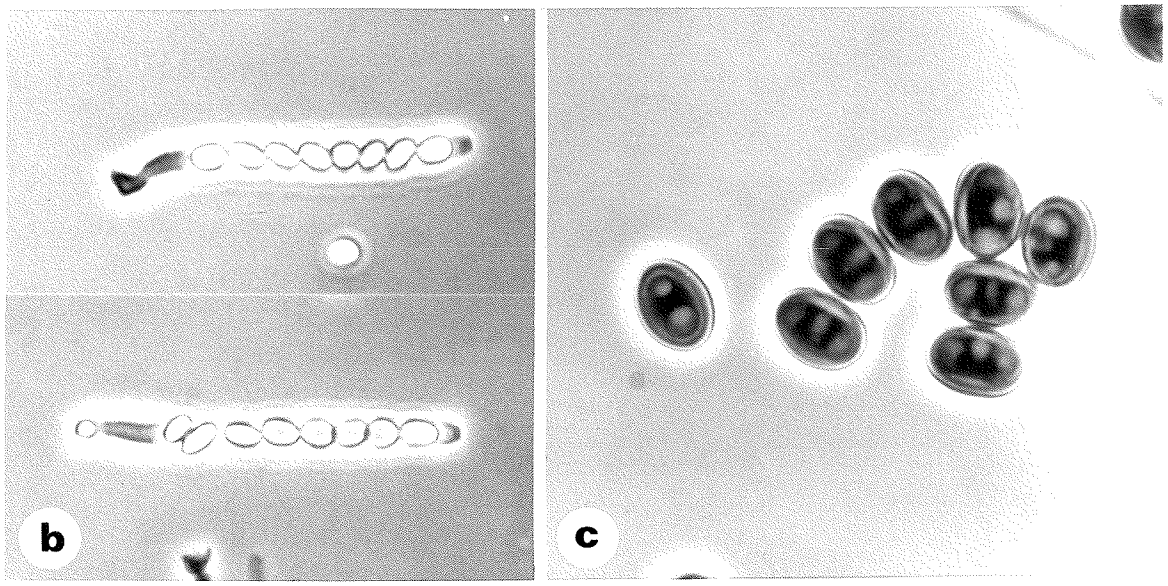
Fig. a. Perithecia (X110).

Fig. b. Asci (x800).

Fig. c. Ascospores (X1850).

Fig. d. Phialides of Phialophora state with conidia (X1850).

a



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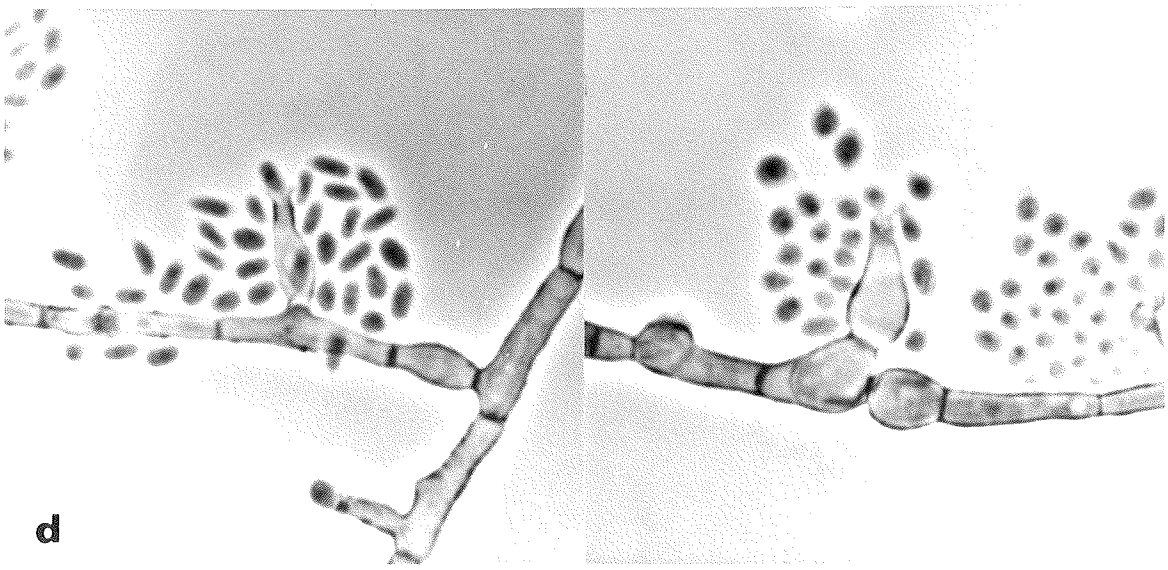


d

infected, wounded or senescent tissues.

Schol-Schwarz (1970) reported that Phialophora luteo-viridis appeared to be constantly connected with dark spored Conichaeta species such as C. velutina. She found that growing the Phialophora states of Coniochaeta spp. on 2% malt extract agar for two weeks at 25°C then exposing them to black light (12 hrs per day) for two or more weeks at 25°C, and finally growing them at 5°C for 1-2 weeks would induce perithecia to form. In this study, the isolate from New Zealand would only form perithecia in vitro when a variation of the above procedure was carried out (see methods and materials). When perithecia were formed, they always occurred in great numbers grouped together and the dark coloured ascospores were ejected from these in such large numbers that they covered the colony, adding to the latter's dark appearance. Mahoney and La Favre reported that forcible discharge of ascospores occurs in C. velutina on agar cultures of wood inhabiting isolates.

When observed using light microscopy no distinct apical pores were visible in the asci. Munk (1957) and Taylor (1970) could not discern a distinct apical ring while Mahoney and La Favre (1981) report the periapical ring was obscure to indistinct.

(c) Wood Staining Coelomycetes

11. Cytospora sp. Plate XII, Figs. a-d.

Colonies attaining a diameter of 64 mm in 7 days at 20°C

on 2% malt agar. Colony floccose, occasionally with an appressed centre; white initially, turning very dark brown and finally black; edge of colony remaining white. Pycnidia superficial to partly embedded in the substrate. Bases black; of various shapes; 200-1,100 µm in diameter and covered with hair-like hyphae. Necks straight or curved, black, 160-1,000 µm in length, 65-270 µm wide at base and 55-90 µm wide at the tip immediately below the apex. Ostiole surrounded by a proliferation of short hyphal elements. Pycnidial interior multilocular, each locule lined with simple to rarely irregularly branched conidiophores. Conidiogenous cells entero-blastic, phialidic, determinate, hyaline. Phialospores hyaline, one-celled, allantoid, 3.0-7.0 x 0.8-1.0 µm.

NEW ZEALAND HOST: Eucalyptus sp.

CULTURES ISOLATED: R 198 (a) isolated from Eucalyptus sp., Riverhead Forest; R 199 (a) isolated from Eucalyptus sp., Riverhead Forest.

Cytospora spp. have been previously reported as staining organisms by Kress et al. (1925), Rumbold (1929), Fritz (1952), and Venn (1972). The first three workers were unable to identify their organisms to species, while Venn only tentatively applied a name.

As Sutton (1980) points out, Cytospora spp. are the anamorphs of Valsa spp. More than 400 spp. have been described for the most part on the basis of physiological specialization (i.e.host substrate rather than

Plate XII

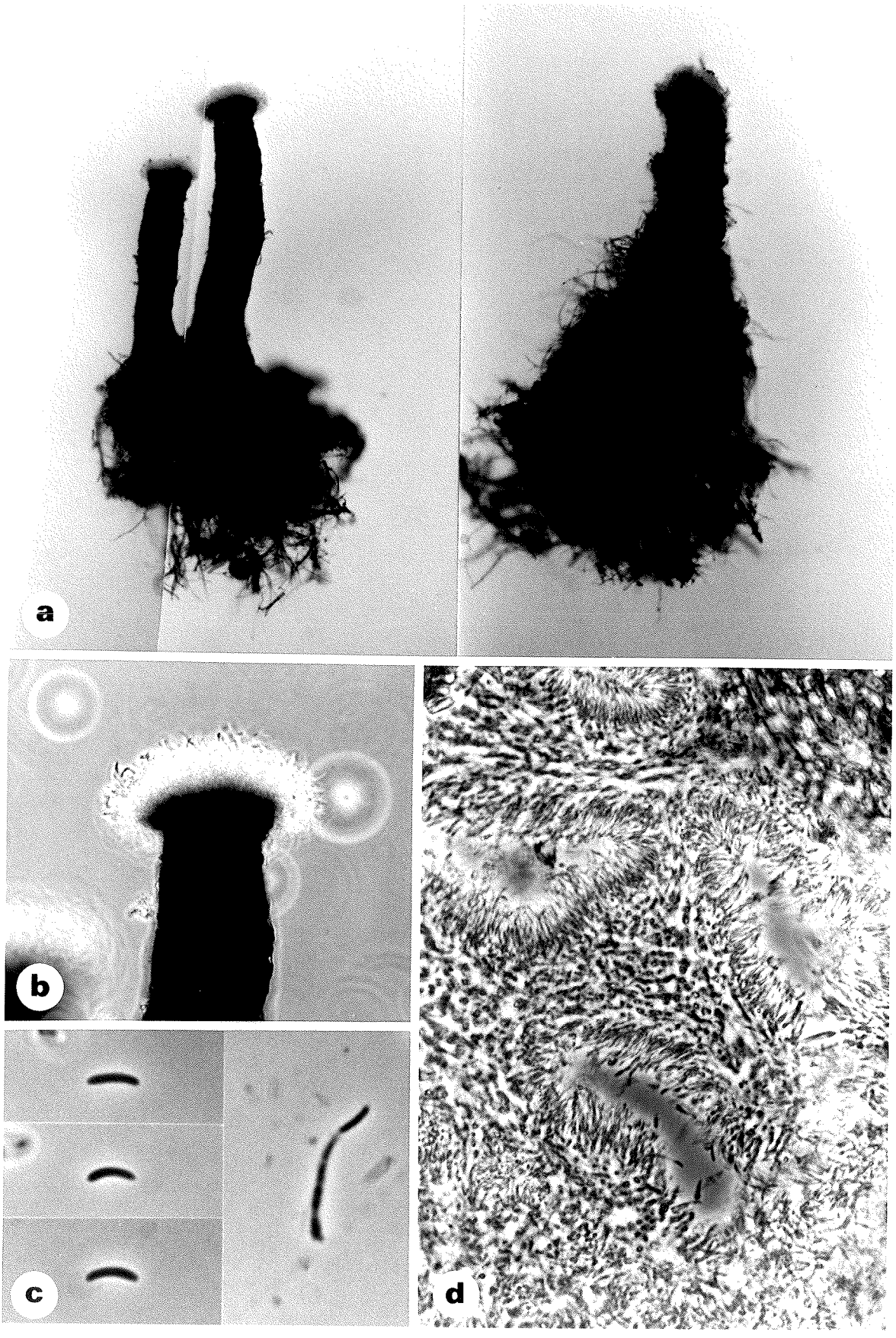
Cytospora sp.

Fig. a. Pycnidia (X95).

Fig. b. Apex of the pycnidial neck surrounded by a fringe of hyphal elements (X245).

Fig. c. Conidia and conidiogenous cell with conidium (X1850).

Fig. d. Longitudinal section of locules within a pycnidium (X610).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d

on morphological differences. Therefore as Fritz pointed out, until the genus is monographed it would be futile to attempt a specific identification of various isolates placed under the generic name Cytospora Ehrenb. ex Fr. However, Zentmyer (1941) suggested that the confused taxonomy of the genus Cytospora could be clarified by a revision of the genus along the lines proposed for the genus Fusarium Link ex Fr. by Snyder and Hansen (1940). Zentmyer felt that the structure of the stroma would be one of the primary morphological characters in formulating species while physiological differences would serve as bases for designating forms. To date, no one has accepted the challenge offered by this proposal.

12. Fusicoccum cf. tingens Goid., R. Staz. Pat. Veg. Bol., Rome, n.s. 16: 267, 1936 Plate XIII, Figs. a-e.

Colonies attaining a diameter of 90 mm or more in 7 days at 20°C on 2% malt agar. At first appressed, then turning floccose; hyaline, becoming light olive-brown and finally very dark-brown; older colonies appearing grey in colour. Pycnidia developing superficially on the substrate; black, columnar in shape, up to 8 mm in length, and up to 2 mm in diameter. Interior multilocular, each locule lined with hyaline and holoblastic conidiogenous cells. Conidia hyaline, one-celled, oval to fusiform in shape, base truncate with a prominent skirt; 13.0-17.0 x 4.0-6.0 µm.

Chlamydospores rare but when present globose to ellipsoidal, pigmented and produced in chains; 4.0-6.0 x 5.0-8.0 µm.

NEW ZEALAND HOSTS: Cupressus macrocarpa Hartw., Pinus radiata D. Don.

CULTURES ISOLATED: R 50 (b) isolated from Cupressus macrocarpa, Woodhill Forest; R 106 isolated from Cupressus macrocarpa, Woodhill Forest; R 108 (c) isolated from Cupressus macrocarpa, Woodhill Forest; 111 (b) isolated from Cupressus macrocarpa; R 116 (b) isolated from Pinus radiata, Woodhill Forest.

This species of Fusicoccum Corda, which stained wood more intensively than any other organism isolated during this study, has many similarities with, but is not identical to, the fungus described and illustrated by Goidanich (1936c) as Fusicoccum tingens. F. tingens, which was isolated from Pinus pinea L., was described as a new species based on the dimensions of the spores, characteristics of the pycnidia and the ability of the mycelium to stain wood; no other species of Fusicoccum able to stain wood had been recorded from conifers. Goidanich noted that fertile pycnidia did not form readily on either wood or on agar media; the great majority of structures which did form being infertile. Only in very old malt agar cultures, and then only in very small numbers, did ripe pycnidia form. A similar observation was made during this study with these New Zealand isolates rarely producing pycnidia in older cultures and then many of them were sterile. However, when grown under black light (see methods and materials) pycnidial formation was stimulated, although still not greatly.

Plate XIII

Fusicoccum cf. *tingens* Goidanich

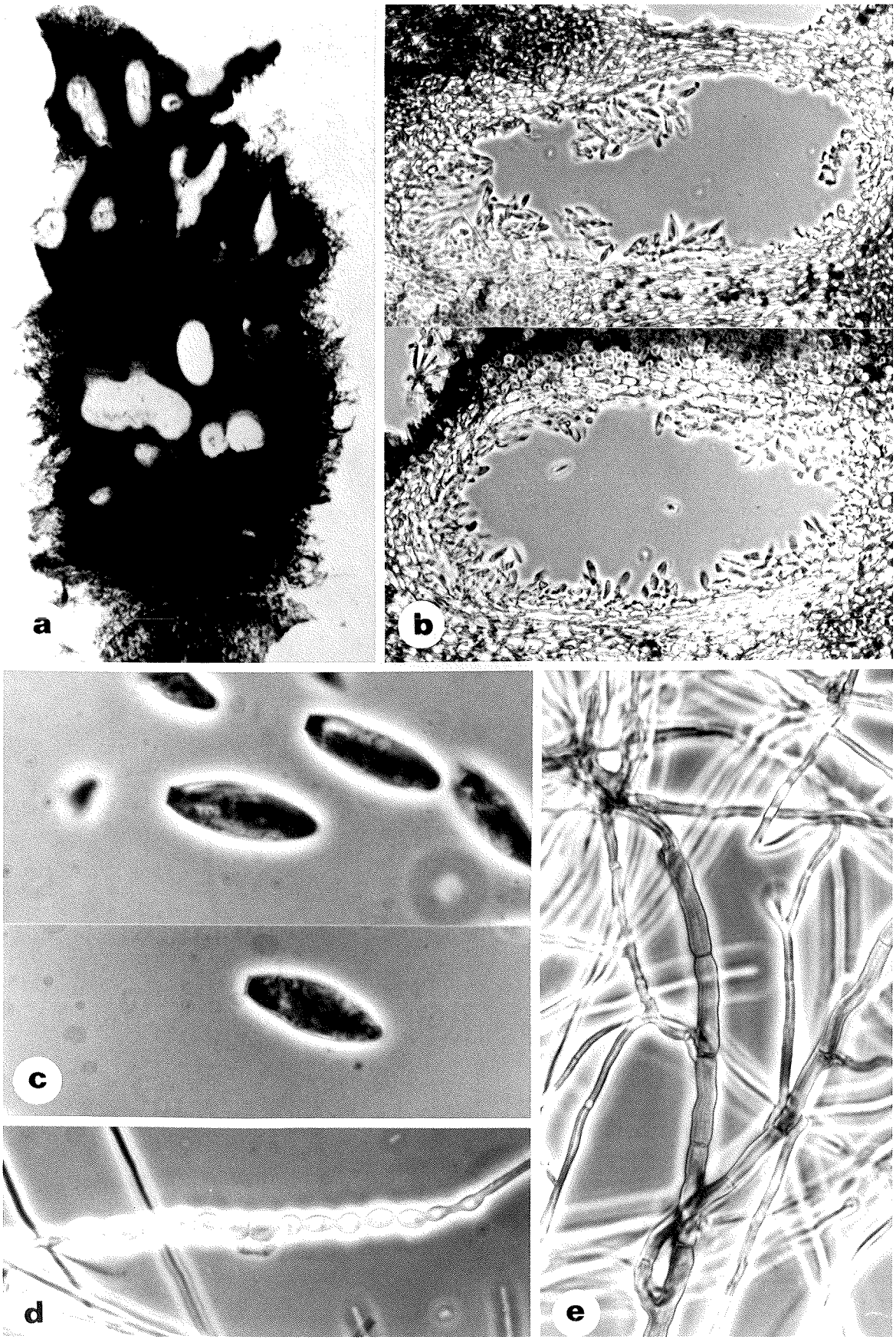
Fig. a. Pycnidium (longitudinal section) (X40).

Fig. b. Sectional view of pycnidial locules with conidiogenous cells (X270).

Fig. c. Conidia (X1650).

Fig. d. Chain of chlamydospores (X680).

Fig. e. Hyphal elements (X680).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Sectioning some of these pycnidia revealed the presence of conidia similar in shape to that of Goidanich's organism except that the New Zealand isolates had smaller conidia (13.0-17.0 x 4.0-6.0 µm vs. 20.5-24.0 x 6.0-7.0 µm for Goidanich's isolate).

Butcher (1968) recorded an unidentified fungus isolated from a Nothofagus sp. in New Zealand which was the main cause of wood staining in his studies. It was somewhat similar to the organism isolated in this present study, e.g. similar growth rate, presence of intercalary to terminal chlamydospores, and production of columnar pycnidia (up to 1,200 µm high x 300-400 µm wide) in older cultures which were mostly sterile. However Butcher (1968) found the conidia to be allantoid which indicates that the organism is not a member of the genus Fusicoccum.

As there are more than 200 taxa assigned to Fusicoccum (Sutton, 1980) and very few of these have been adequately studied, it seems most appropriate to tentatively refer the Fusicoccum sp. isolated during this present study as being the same as that described by Goidanich.

13. Phoma sp. Plate XIV, Figs. a-d.

Colonies attaining a diameter of 64 mm in 12 days at 20°C on 2% malt agar. Colony appressed to slightly floccose; at first hyaline, becoming reddish-brown and finally turning dark olive-grey in the centre, but remaining hyaline to white around the

edges; often sectored with the formation of pycnidia giving a peppered appearance to the colony. Conidiomata pycnidial, developing superficially on the mycelium; brown to black in colour. Bases globose to ovoid; 130-245 µm in diameter; smooth. Necks short, straight, cylindrical, brown to black, 25-32 µm long, 53-69 µm wide; or absent. Ostiole surrounded by irregular extensions of hyphal elements. Pycnidia unilocular; lined with hyaline, enteroblastic conidiogenous cells. Phialospores hyaline, one-celled, oblong to slightly ovoid with obtuse apices and occasionally curved; 3.5-5.0 x 1.0-2.0 µm; collecting in a slime drop at the top of the pycnidium.

NEW ZEALAND HOST: Larix sp.

CULTURE ISOLATED: R 154 (c) isolated from Larix sp., Waiotapu Forest.

According to Sutton (1980), the genus Phoma Sacc. has traditionally been used for simple stem-inhabiting pycnidial fungi with small hyaline, unicellular conidia. Species separation has been based on minor morphological differences and the nature of the host substrate. Because of this, some 2,000 species have now been described and it is unlikely that this reflects true species relationships. For this reason it is hoped that in vitro studies of this group under standardized conditions (e.g. Boerema, 1976) will allow for a scheme of identification of Phoma spp. similar to that developed for the genus Fusarium by Booth (1971). However, despite the advances made to date in the taxonomy of this genus,

Plate XIV

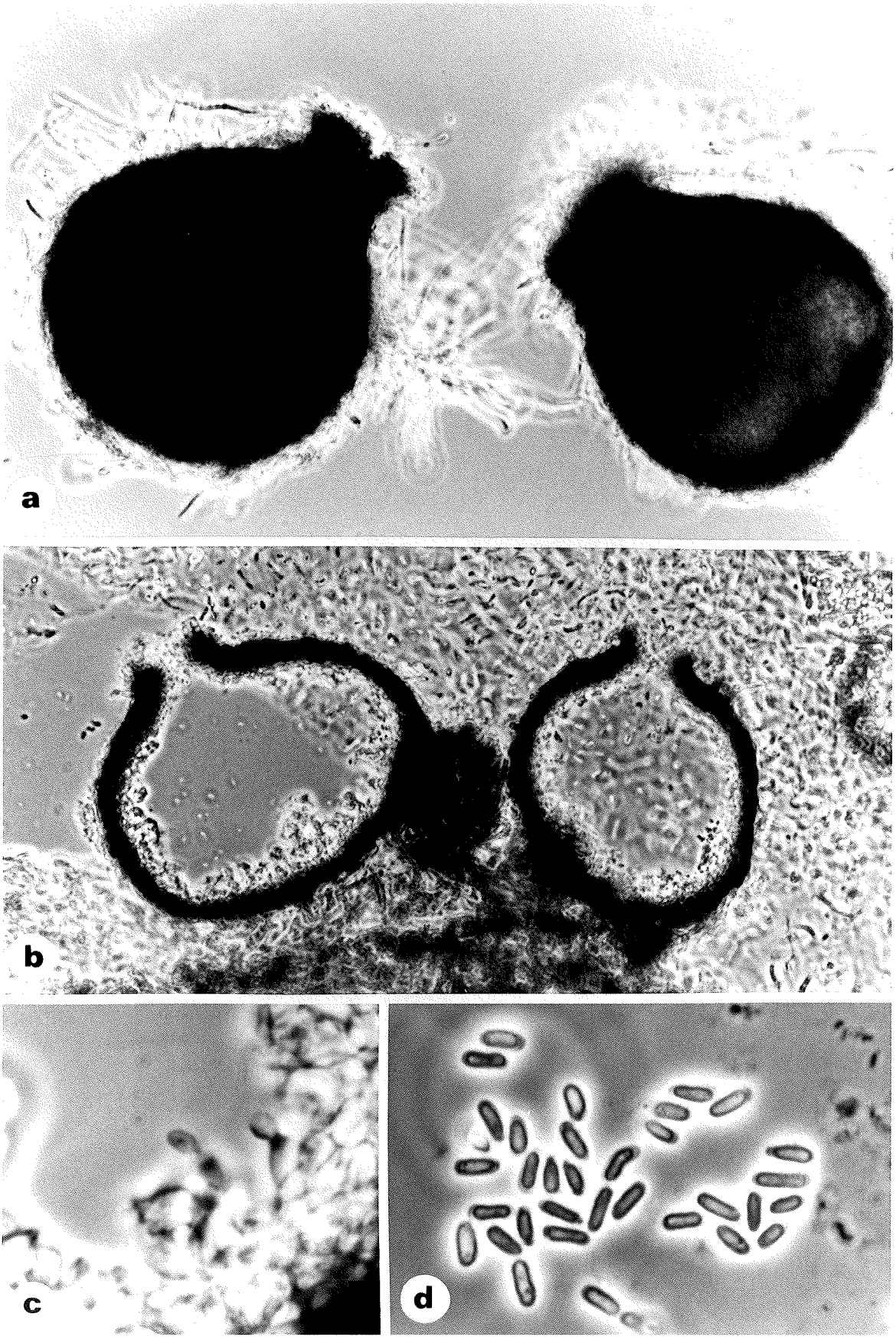
Phoma sp.

Fig. a. Pycnidia (X300).

Fig. b. Longitudinal section of pycnidia (X300).

Fig. c. Conidiogenous cells (X1900).

Fig. d. Conidia (X1900).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it was not possible to identify the Phoma isolate from New Zealand to species.

(d) Wood Staining Hyphomycetes

14. Aureobasidium pullulans (de Bary) Arnaud var. pullulans Hermanides-Nijhoff, Stud. Mycol. 15: 158, 1977 Plate XV, Figs. a-e.

For a complete synonymy see Hermanides-Nijhoff (1977).

Colonies attaining a diameter of 42-50 mm in 12 days at 20°C on 2% malt agar. Colony very effuse, slimy; hyaline at first, then turning pinkish white and finally becoming pale brown with dark brown to black sectors. Hyphae very sparse, turning dark brown and fragmenting into dark-coloured chlamydospore-like structures. These dark-coloured cells, which appear in great numbers measure 7.5-13.1 x 2.75-6.0 µm. Conidia are produced directly from thick, dark-coloured mycelium from closely knit loci and give the appearance of a palisade layer. Conidia are also infrequently produced as sympodulospores from semi-macronematous conidiogenous cells which arise from thin hyaline hyphae. Conidia hyaline; one-celled; ellipsoidal to oval, with or without a basal scar; smooth; 4.0-8.0 x 2.0-3.0 µm.

NEW ZEALAND HOST: Cupressus macrocarpa Hartw.

CULTURE ISOLATED: R 51 (cii) isolated from Cupressus macrocarpa, Woodhill Forest.

Plate XV

Aureobasidium pullulans (de Bary) Arnaud var. pullulans Hermanides-Nijhof.

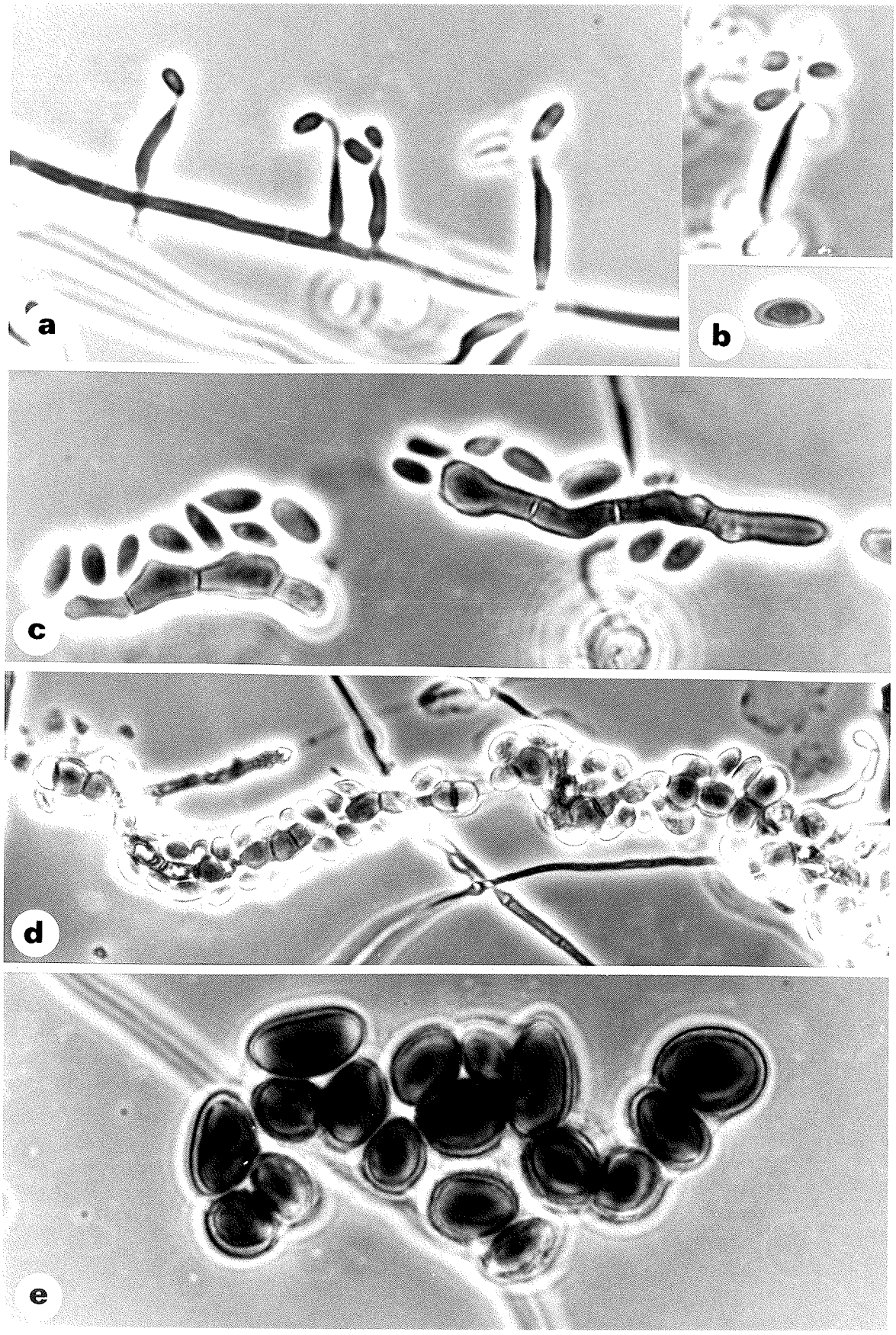
Fig. a. Conidiogenous cells with sympodulospores (X1900).

Fig. b. Conidium (X1900).

Fig. c. Sparse hyphae with direct production of conidia (X1900).

Fig. d. Disarticulation of hyphae and conversion into budding cells (X760).

Fig. e. Budding cells (X1900).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As one of the black yeasts, A. pullulans has created so much confusion in its taxonomy that even the most experienced and talented fungal taxonomist often has difficulty in identifying it. The long list of synonyms by Cooke (1962) and the shorter one by Hermanides-Nijhoff can attest to that. Melin and Nannfeldt (1934) found that the number of discernible strains they isolated seemed to be almost unlimited and almost every new isolation showed discrepancies from those previously isolated.

A. pullulans has been previously isolated as a wood staining organism by Melin and Nannfeldt in Sweden; Davidson (1935) in the United States; and Eckersley (1934) in Australia; the latter author reported it as Hormonema dematioides Lagerb. & Melin [H. dematioides = A. pullulans fide Cooke (1962)]. These workers, and others, observed the production of dark coloured yeast-like cells which proliferated to cover the colony and darken it over time. Observations on the New Zealand isolate indicate that these cells arise from the fragmentation of the sparse hyphal elements and then reproduce by budding.

The dark-coloured yeast-like cells, method of production of conidia directly from the mycelium, and colony characteristics of the New Zealand isolate agree well with the described characteristics of A. pullulans. However, the infrequent production of sympodulospores on short conidiogenous cells has never been recorded previously for this fungus. Hoog (1977) erected the genus Leptodontium de Hoog [now Leptodontidium de Hoog since Leptodontium de

Hoog (1977) is a homonym of Leptodontium (C. Huell.) Hamp. ex Lindb., a moss genus] of which some species possess sympodial conidiogenous cells similar to what was found in the New Zealand isolate of A. pullulans. This sympodial production of conidia occurred only rarely and only when the organism was grown out onto glass slides. Although it is possible a contaminant might have been responsible for the elements producing the sympodulospores, cultures did not otherwise show signs of contamination. Due to the extremely heterogenous nature of this fungus, it would not be surprising if this is indeed a type of conidial formation to be found in A. pullulans.

15. Cladosporium cladosporioides (Fres.) de Vries, Contribution to the Knowledge of the Genus Cladosporium Link ex Fr.: 57, 1952 Plate XVI, Fig. a.

≡ Penicillium cladosporioides Fres., Beitr. Mykol. 3: 22, 1863.

≡ Hormodendrum cladosporioides (Fres.) Sacc., Michelia 2: 148, 1880.

Colonies attaining a diameter of 30-34 mm in 7 days at 20°C on 2% malt agar; appressed except for the conidiophores which give colony felt-like appearance; olive grey in colour. Conidiophores mononematous; macronematous and micronematous; with a light to dark-coloured stipe; 85-285 µm long, 2.5-5.0 µm wide; stipe evenly wide along entire length. Ramoconidia 0-1 septate; up to 30 µm long; light-coloured. Conidia produced blastically in long branching

chains; pale olive brown in colour; one-celled, ellipsoidal to limoniform; 4.0-8.0 x 2.0-4.0 µm.

NEW ZEALAND HOSTS: Eucalyptus sp., Larix sp.

CULTURES ISOLATED: R 23 (a) isolated from Larix sp., Waiotapu Forest; R 25 (b) isolated from Larix sp., Waiotapu Forest; R 155 (b) isolated from Larix sp., Waiotapu Forest; R 156 (b) isolated from Larix sp., Waiotapu Forest; R 183 isolated from Eucalyptus sp., Riverhead Forest.

Ellis (1971) mentions that C. cladosporioides is a very cosmopolitan species, occurring as a secondary invader on many different plants and that it has been isolated from air, soil, textiles, etc. It was recorded as a staining organism in the first surveys of wood staining fungi ever done (Hedgcock 1906; Munch 1907) and since then has been found in most regions surveyed.

As a genus, Hormodendrum Bon. was distinguished from Cladosporium Link ex Fr. by having non-septate conidia while the latter genus possessed septate conidia. Hedgcock (1906) and Robak (1932) recognized that the septate branches of C. cladosporioides could indeed be considered as conidia because as Robak reported, they can germinate and form new colonies. Vries (1952) reduced Hormodendrum to synonymy with Cladosporium, and this is supported by the fact that most isolates of Cladosporium do produce a high percentage of non-septate conidia (Onions et al., 1981).

Plate XVI

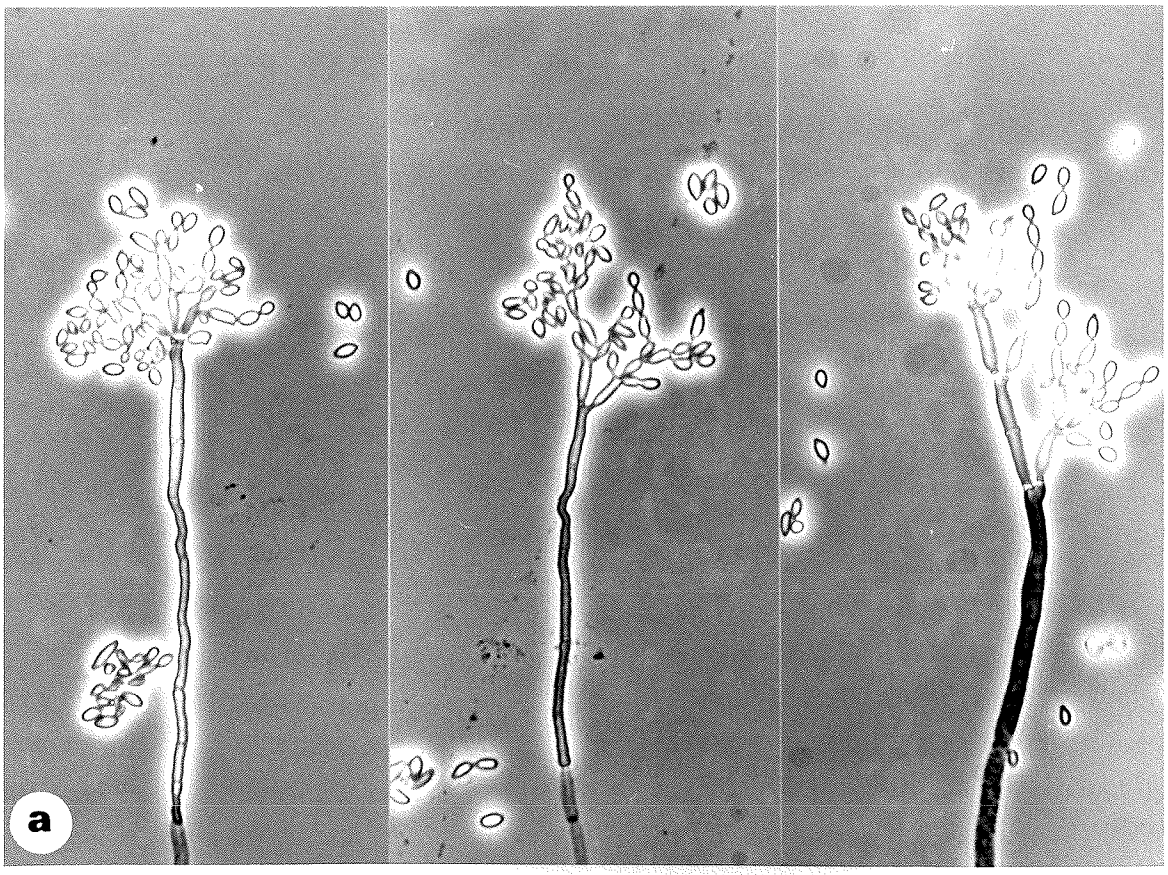
Cladosporium cladosporioides (Fres.) de Vries

Fig. a. Conidiophores (X650).

Cladosporium tenuissimum Cooke

Fig. b. Conidiophore (X100).

Fig. c. Conidiogenous head (X720).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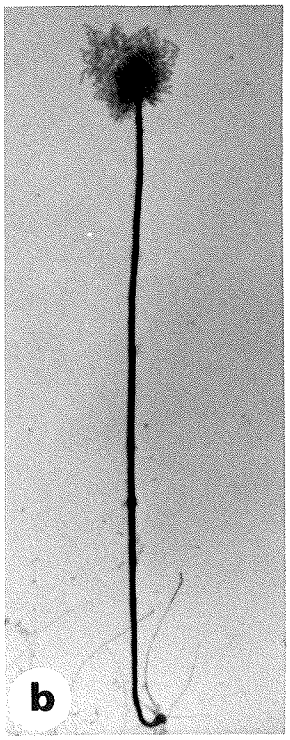
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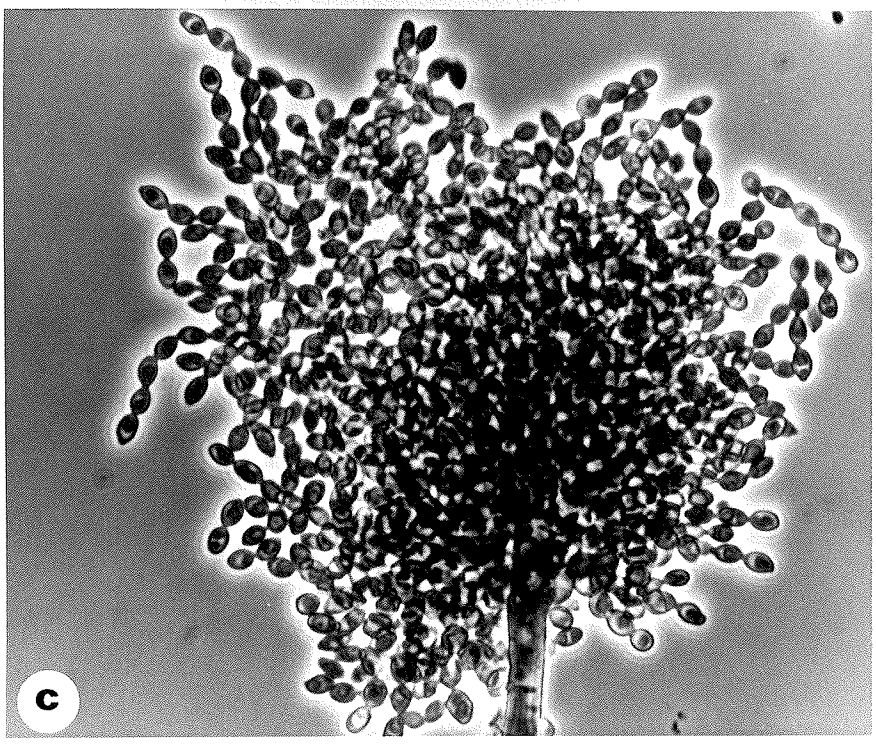
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16. Cladosporium tenuissimum Cooke, Grevillea 6: 140, 1878
Plate XVI, Figs. b-c.

Colonies attaining a diameter of 36-38 mm in 12 days at 20°C on 2% malt agar. Colony felt-like; light yellowish-brown at first then changing to olive in colour. Conidiophores mononematous, macronematous, with a parallel sided dark coloured stipe; 520-970 µm long and 5.0-7.6 µm wide; straight or slightly curved. Conidia produced as blastospores in long, branched chains. Conidia pale brown to olivaceous-brown; one to two celled; cylindrical; ellipsoidal; 4.0-16.0 x 2.0-4.5 µm.

NEW ZEALAND HOST: Podocarpus sp.

CULTURE ISOLATED: R 142 (b) isolated from Podocarpus sp., Minginui Forest.

Ellis (1976) records C. tenuissimum as being world wide in distribution, having been isolated from 40 different plant species. During this present study, this staining organism was isolated only once from New Zealand; it has not been recorded previously as having a staining capability.

17. Exophiala jeanselmei (Langer.) McGinnis & Padhye var. jeanselmei de Hoog, Stud. Mycol. 15: 108, 1977 Plate XVII, Figs. a-e.

≡ Torula jeanselmei Langer., Ann. Parasit. hum. comp. 6: 396, 1928.

≡ Pullularia jeanselmei (Langer.) Dodge, Med. Mycol. p. 675, 1935.

≡ Phialophora jeanselmei (Langer.) Emmons, Arch. Path. 39: 368, 1945.

≡ Exophiala jeanselmei (Langer.) McGinnis & Padhye, Mycotaxon 5: 345, 1977.

Colonies attaining a diameter of 10-14 mm in 12 days at 20°C on 2% malt extract agar; velvety to floccose; at first hyaline, then turning reddish-grey and finally reddish-brown. Hyphae producing short intercalary conidiogenous cells which in turn produce conidia terminally or laterally: (1) on small, tapering, lateral peg-like outgrowths in an apparent holoblastic manner; (2) occasionally on such pegs which appear to have proliferated sympodially; (3) from indistinctly percurrently proliferating phialides; or (4) non-proliferating phialides with small but distinct collarettes. Conidia hyaline, subhyaline, to light brown; one-celled; ellipsoidal or clavate to slightly obovate, with an inconspicuous hilum; 3.0-7.0 x 1.2-3.5 µm (3.2-4.4 x 1.2-2.2 µm; de Hoog, 1977). Secondary conidia frequently formed on detached primary conidia.

NEW ZEALAND HOST: Pinus radiata D. Don.

CULTURE ISOLATED: R 73 (a) isolated from Pinus radiata, Tairua Forest.

Species of the genus Exophiala Carmichael have conidia pro-

Plate XVII

Exophiala jeanselmei (Langer.) McGinnis & Padhye
var. jeanselmei de Hoog.

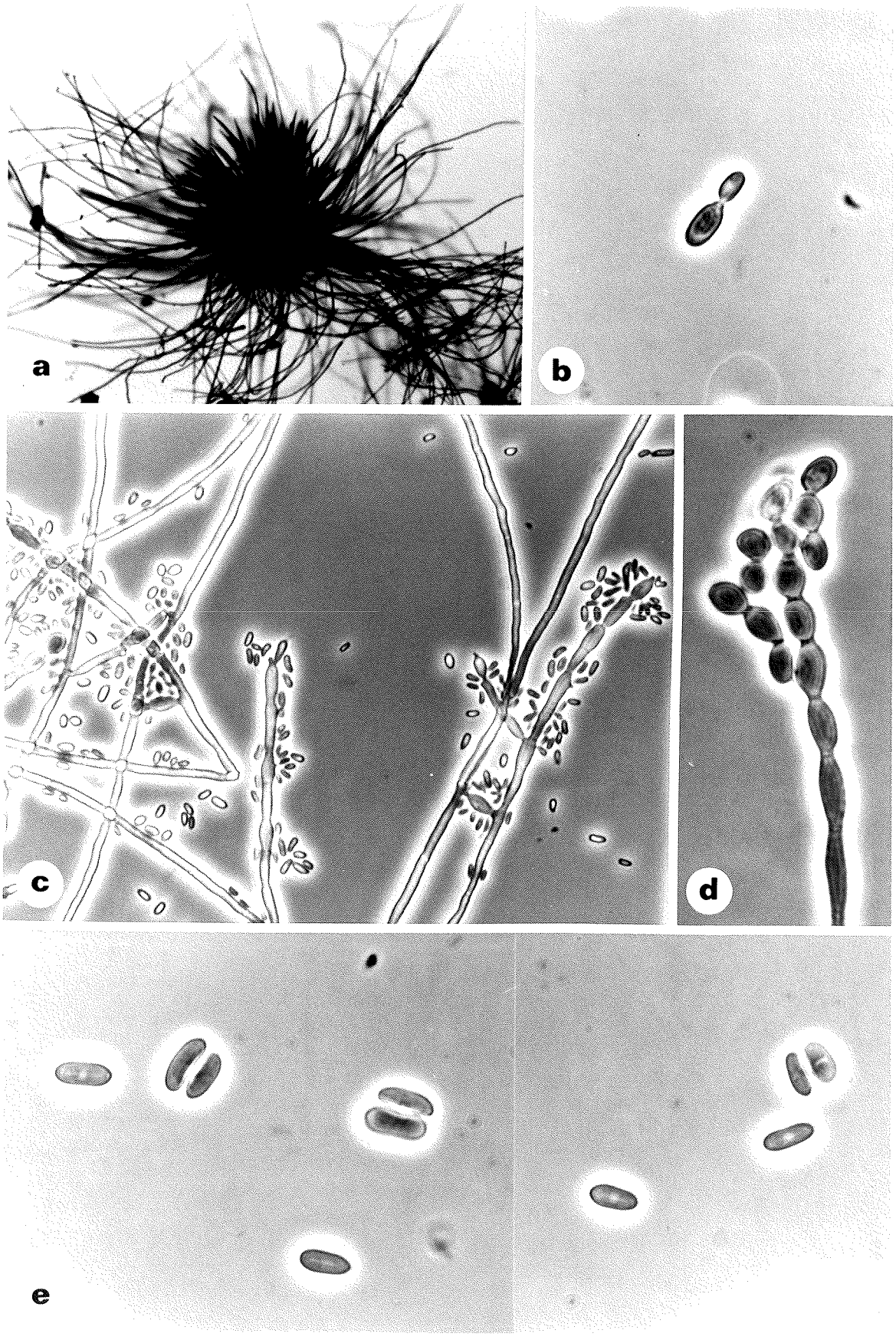
Fig. a. Protoperithecium (X105).

Fig. b. Budding conidium (X1700).

Fig. c. Conidiophores with conidia (X680).

Fig. d. Torulose mycelium (X1700).

Fig. e. Conidia (X1700).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duced percurrently through the scars of previously liberated conidia and budding cells as well as phialides may be present.

E. jeanselmei was divided by de Hoog (1977) into three varieties based on morphological differences but he mentions that these varieties are only gradationally distinct and feels that some strains become similar to var. jeanselmei after prolonged culture in vitro.

When old cultures of the New Zealand isolate of E. jeanselmei var. jeanselmei were exposed to black light (see methods and materials) immature ascocarps formed (Fig.a, Plate XVII), but these never matured. These structures were black, globose to ovoid, 100-210 µm high and 80-115 µm in diameter at the base and covered with long dark-coloured hairs which were 95-140 µm in length and 4.5-6.0 µm in width at the base. The only record of a teleomorphic state occurring in a species of Exophiala is that recorded for Exophiala mansonii (Castell.) de Hoog whose teleomorph was described by Schol-Schwarz (1968) as Dictyotrichiella mansonii Schol-Schwarz. Schol-Schwarz reported that D. mansonii produced ascocarps which are globose to subglobose, 70-100 µm in diameter and covered with dark-brown septate setae 37-76(120) x 3-8 µm and possessed eight muriform ascospores in each bitunicate ascus. She found that these ascocarps developed within 8 days when the fungus was cultivated at 25°C in tubes with a stalk of a Lupinus sp. incorporated into oatmeal agar. This sexual state developed in only one strain she studied and the sexual state is still only known in culture.

Schol-Schwarz believed Torula jeanselmei (≡ Exophiala

jeanselmei var. jeanselmei) to be synonymous with Rhinocladiella mansonii (Castell.) Schol-Schwarz (≡ Exophiala mansonii) while de Hoog felt that they were separate entities. In addition to this, Nannfeldt and Melin (1934) reported the presence of small sclerotial bodies in Trichosporium heteromorphum Nannf. [≡ Exophiala jeanselmei (Langer.) McGinnis & Padhye var. heteromorpha (Nannf.) de Hoog] as did de Hoog for Exophiala jeanselmei (Langer.) McGinnis & Padhye var. lecanii-corni (Benedek & Specht) de Hoog. Since the term sclerotial body is often used by many workers to refer to proto-perithecia, they may have noted structures similar to those reported on herein.

It was not possible during this study to induce the immature ascocarps (ascostromata?) to mature so one cannot speculate whether they represent a developmental stage of a Dictyotrichiella Munk. Possibly this organism is heterothallic and requires the presence of two mating types before these immature ascocarps will mature or there could be another explanation. For example: (1) the isolate was a mutant form with a genetic block somewhere in ascus formation; (2) the isolate contained a physiological vitamin deficiency necessary for the formation of ascospores; and (3) an undetected contaminant was blocking ascospore formation (Hinds and Davidson, 1967).

18. Fusarium sp. Plate XVIII, Figs. a-e.

Colonies attaining a diameter of 70-80 mm in 12 days at 20°C on 2% malt agar. Colony floccose; at first white, then turning pink and finally weak red. Microconidial conidiogenous cells borne on simple to irregularly branched mononematous, semi-macronematous conidiophores. Conidiogenous cells enteroblastic, hyaline, variable in length but with a discrete collarette; cylindrical to subulate; hyaline. Microconidia hyaline; one-celled; ellipsoidal, oval to ovoid; 4.0-13.0 x 2.75-6.75 µm. Macroconidia borne on more elaborately branched conidiophores (penicillately branched), in sporodochia. Conidiogenous cells enteroblastic, ovoid to obpyriform with a discrete collarette. Macroconidia hyaline; inequilaterally curved, with a prominent foot-cell; 3-5 celled; 22.5-45.0 x 3.75-7.5 µm.

NEW ZEALAND HOST: Pinus elliotii Engelm.

CULTURE ISOLATED: 67 (a) isolated from Pinus elliotii, Tairua Forest.

This fungus, which caused a red staining in wood, corresponds closely to the organism Hedgcock (1906) called Fusarium roseum Link. Indeed his description and illustrations strongly suggest that the fungus he studied was probably the same organism. However, the name F. roseum is no longer accepted, having been recognized as comprising a heterogenous mixture of different Fusarium spp. (see Booth, 1971) and it was not possible to assign, with confidence, a specific epithet to the New Zealand isolate; it quickly lost its

Plate XVIII

Fusarium sp.

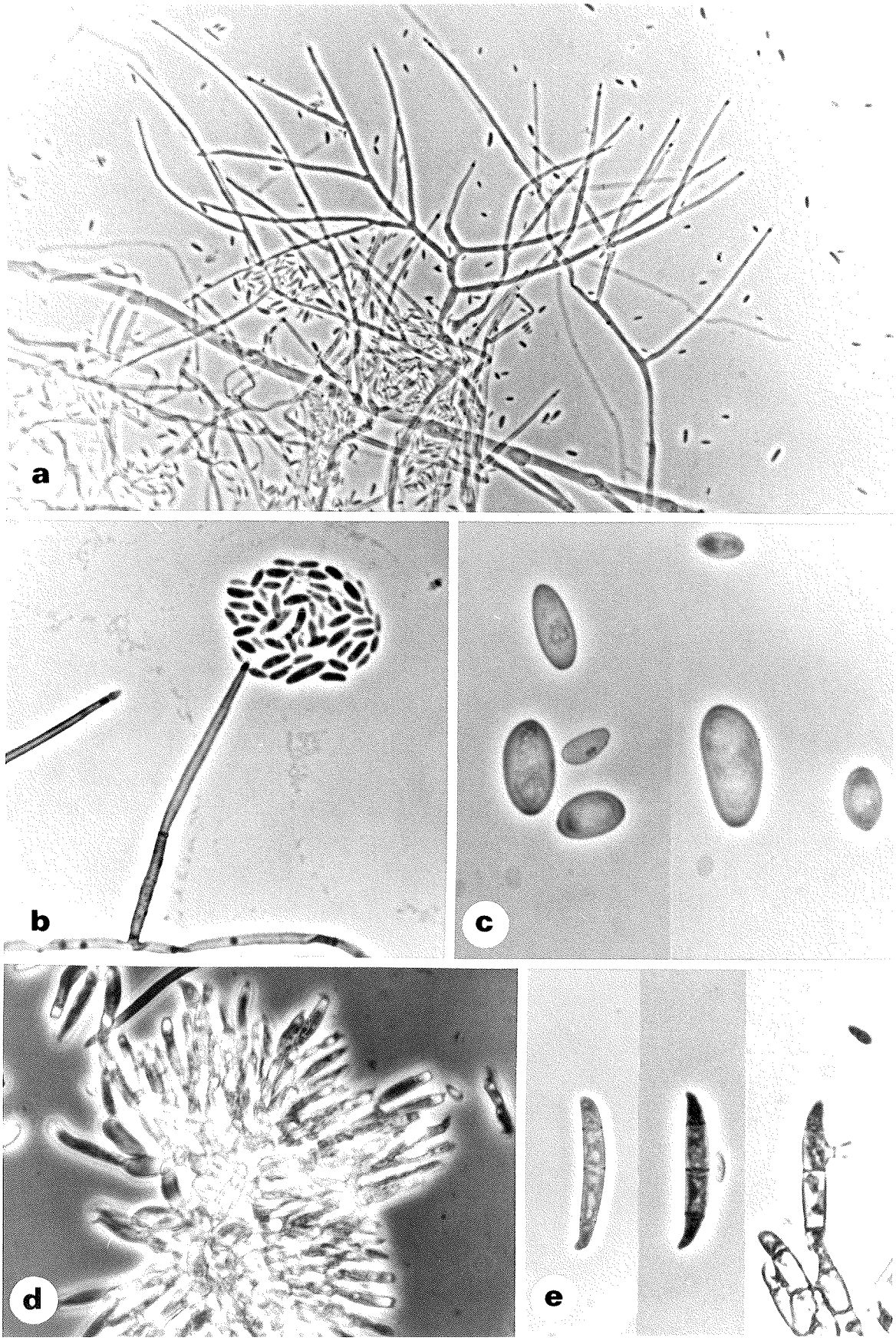
Fig. a. Microconidiophores (X315).

Fig. b. Phialide with microconidia (X680).

Fig. c. Microconidia (X1650).

Fig. d. Macroconidiophores (X680).

Fig. e. Macroconidia; conidium on far right showing phialide (X880).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ability to produce macroconidia in culture and macroconidia are the key taxonomic character in identification of the Fusaria.

Brief initial observations made on freshly isolated cultures showed that macroconidial formation was sparse and only occurred after considerable periods of time (Hedgcock found that the macroconidia in his organism were sparsely found only in older portions of the colony). The macroconidia were produced on sporodochia and they often germinated to produce phialides (Fig.e, Plate XVIII) from which microconidia developed. This phenomenon was also observed in an isolate of Fusarium culmorum (W.G. Smith) Sacc. which Booth (1971) implies is a common occurrence for that species.

Other reports of Fusarium spp. causing red staining in wood are by Goidànich (1936b) and Batra and Lichtwardt (1962). Goidanich reported on Fusarium javanicum Koord. [= Fusarium solani (Mart.) Sacc. fide Booth (1971)] found staining Populus canadensis Moench in Italy, while Batra and Lichtwardt investigated Fusarium reticulatum Mont. var. negundinis (Sherb.) Wr. [= Fusarium heterosporum Nees ex Fr. fide Booth (1971)] staining Acer negundo L. in Kansas. Batra and Lichtwardt report that their fungus was regularly associated with the larvae of beetles and was sometimes a contaminant in the galleries of the ambrosia beetle Xyleborus saxeseni Ratzberg.

19. Hyalopesotum pini sp. nov. Plate XIX, Figs. a-d.

Coloniae in agaro cum extracto malti (2%) praeter conidiophora adpressae, primo hyalinae, post 12 dies avellanescentes. Conidiophora macronemata mononemataque synnematave; stipes conidiophori mononemati hyalinus avellanescens, 50-240 µm longus, 3.8-7.5 µm latus basi, 3.0-3.8 µm latus proxime infra zonam conidiogenam; stipes conidiophori synnemati hyalinus brunnescens 60-250 µm longus, 12.5-140 µm latus. Conidia, sympodulosporae vel phialosporae, hyalina, oblonga vel clavata, versus unum extremum decrescentia 3.0-7.0 x 1.0-2.2 µm.

TYPUS: R 88 (a) sejunctus ex Pino radiata D. Don, ex sylva Tairuae, Nova Zelandia. J. Reid legit May 29, 1982.

Colonies attaining a diameter of 42-46 mm in 12 days at 20°C on 2% malt agar; appressed except for upright conidiophores; hyaline at first, but becoming light yellowish brown. Conidiophores macronematous and mononematous to synnematous. Mononematous conidiophores branched towards the apex forming a stipe and a head; stipe straight to flexuous; hyaline to light brown; head composed of several series of branches with spores accumulating in a slimy head. Stipe 50-240 µm in length, 3.8-7.5 µm wide at the base, 3.0-3.8 µm wide immediately below the conidiogenous zone. Synnemata capped by slimy heads; individual hyphae resembling the mononematous, macronematous conidiophores; hyaline but after prolonged aging becoming brown. Stipe 60-250 µm in length, 12.5-140 µm in width.

Conidiogenous cells either: (1) polyblastic, discrete, sympodial, cylindric to subulate and arranged penicillately or (2) monophialidic, discrete, arranged penicillately, determinate to percurrent, with well defined collarettes which are sometimes flared. Conidia produced either as sympodulospores or phialospores; hyaline, one-celled, oblong to clavate and tapering to one end; ends obtuse; 3.0-7.0 x 1.0-2.2 µm.

TYPE: R 88 (a) isolated from Pinus radiata D. Don, Tairua Forest, New Zealand. Collected by J. Reid May 29th, 1982.

NEW ZEALAND HOSTS: Pinus radiata D. Don, Pinus taeda L.

CULTURES ISOLATED: R 29 isolated from Pinus radiata, Woodhill Forest; R 61 isolated from Pinus radiata, Tairua Forest; R 82 (a) isolated from Pinus radiata, Tairua Forest; R 87 (b) isolated from Pinus radiata, Tairua Forest; R 88 (a) isolated from Pinus radiata, Tairua Forest; R 88 (b) isolated from Pinus radiata, Tairua Forest; R 88 (c) isolated from Pinus radiata; R 122 (a) isolated from Pinus radiata, Woodhill Forest; R 184 (a) isolated from Pinus taeda, Riverhead Forest; R 187 (a) isolated from Pinus taeda, Riverhead Forest; R 187 (b) isolated from Pinus taeda, Riverhead Forest; R 187 (c) isolated from Pinus taeda, Riverhead Forest; R 189 (a) isolated from Pinus taeda, Riverhead Forest; R 189 (b) isolated from Pinus taeda, Riverhead Forest; R 189 (d) isolated from Pinus taeda, Riverhead Forest; R 189 (e) isolated from Pinus taeda, Riverhead Forest; R 189 (f) isolated from Pinus taeda, Riverhead Forest; R 189 (g) isolated from Pinus taeda, Riverhead Forest.

Plate XIX

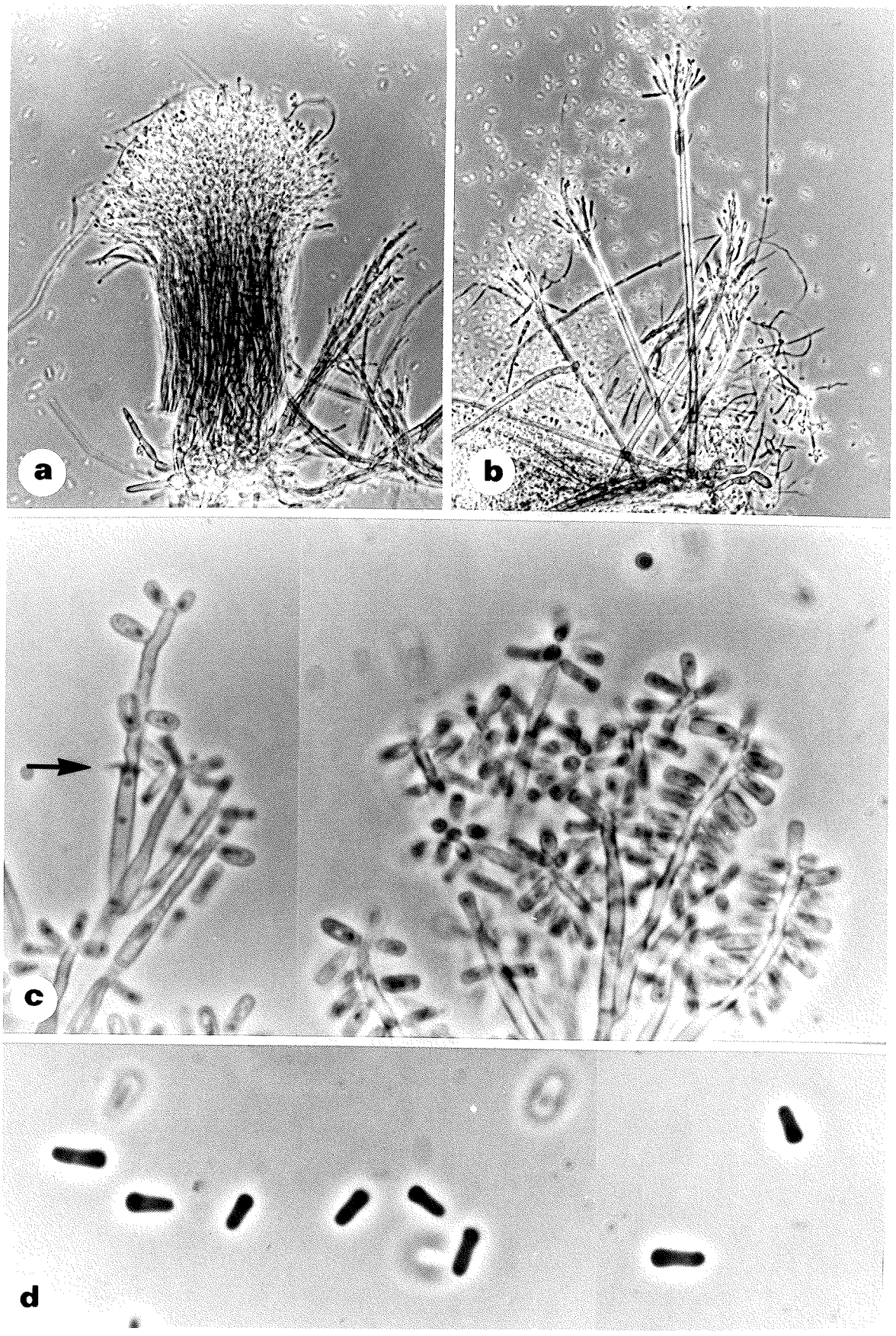
Hyalopesotum pini sp. nov.

Fig. a. Synnema (X265).

Fig. b. Mononematous conidiophores (X265).

Fig. c. Conidiogenous cells; note sympodial conidiogenous cell arising percurrently from a phialide (X1650).

Fig. d. Conidia (X1650).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Hyalopesotum, type species H. introcitrina Upadhyay & Kendr. (anamorph of Ceratocystis introcitrina Olchow. & Reid) was erected by Upadhyay and Kendrick (1975) for those species of synnematous fungi which were similar to Pesotum except that they possessed hyaline to cream-white synnemata. Upadhyay (1981) recognized three species as belonging to this genus, all anamorphs of Ceratocystis spp. (anamorphs of C. arborea Olchow. & Reid and C. araucariae Butin, and H. introcitrina). During the present study it became apparent that Hyalopesotum represents a poorly defined group of organisms for the following reasons. (1) The synnemata of the anamorph of C. arborea can often be pale brown in colour (Upadhyay, 1981). This was also observed in H. pini whose synnemata regularly became brown after prolonged aging. (2) While the anamorph of C. arborea and H. introcitrina only form true synnemata, H. pini produces both mononematous and synnematous conidiophores while the anamorph of C. araucariae can possess up to 5 types of conidial reproduction (de Hoog and Scheffer, 1984). (3) Phialospores and sympodulospores are produced on separate conidiogenous cells on the same conidiophore in the anamorph of C. araucariae (de Hoog and Scheffer, 1984) and this was also discovered in H. pini. In addition, it was found that in H. pini sympodial spore production sometimes occurred on conidiogenous cells which previously had been percurrently phialidic (Fig.c, Plate XIX). Despite these points which argue against character stability in , and thus the integrity of, the genus Hyalopesotum, the New Zealand isolates are assigned here simply because there is

no other repository unless one undertakes an entire revision of the Leptographium and Graphium complexes.

As all previously described species of Hyalopesotum have their teleomorph in the genus Ceratocystis, this is probably also the case with H. pini. Olchowecki and Reid (1974) as well as Upadhyay (1981) found that perithecia of H. introcitrina would only form on wood. This substrate did not induce perithecial formation in H. pini; neither did growing isolates at a cool temperature (9°C), under black light on agar medium containing vitamins, in a mixed culture with Gliocladium roseum, nor mating different isolates in different crosses (in case of heterothallism).

20. Leptodontidium elatius (Mangenot) de Hoog var. elatius de Hoog, Taxon 28: 348, 1979 Plate XX, Figs. a-d.

≡ Rhinocladiella elatior Mangenot, Revue gen. Bot. 59: 57, 1952.

≡ Leptodontium elatius (Mangenot) de Hoog var. elatius de Hoog, Stud. Mycol. 15: 47, 1977.

Colonies attaining a diameter of 6 mm in 12 days at 20°C on 2% malt agar. Colony flocculose; in raised cushion; grey at first, but becoming dark grey. Conidiogenous cells arising from hyphae; long to short; producing conidia in a sympodial fashion; hyaline; the shorter conidiogenous cells producing conidia in a fan-shaped arrangement. Conidia hyaline, one-celled, fusiform to ovoid,

4.0-7.0 x 1.2-3.0 μm. Chlamydospores often produced; dark-coloured; globose, subglobose to oblong and always in chains; each chlamydospore 7.0-8.75 μm in width.

NEW ZEALAND HOSTS: Beilschmiedia tawa (A. Cunn.) Kirk, Pinus radiata D. Don.

CULTURES ISOLATED: R 89 (d) isolated from Pinus radiata, Whangapoua Forest; 100 (b) isolated from Pinus radiata, Whangapoua Forest; 136 (d) isolated from Beilschmiedia tawa, Minginui Forest.

Nannfeldt (in Melin and Nannfeldt, 1934) erected the genus Rhinocladiella Nannf., type species Rhinocladiella atrovirens Nannf. The species of this genus were originally described as producing conidia only on small denticles at the apex of the conidiogenous cells but Schol-Schwarz (1968) in her monograph showed that phialides also could be present. Rhinocladiella elatior was made the type species of the new genus Leptodontium de Hoog (1977), since de Hoog felt it differed from other species of Rhinocladiella in cultural characteristics, including the occurrence of hyaline fertile cells. However, because Leptodontium de Hoog was a later homonym of Leptodontium (C. Muell.) Hamp. ex Lindb., a moss genus, de Hoog (1979) proposed the name Leptodontidium nom. nov. to replace Leptodontium de Hoog.

Based on morphological differences de Hoog (1977) divided the species into two varieties and reported that both varieties of this organism are common on conifers in the northern temperate zone. The New Zealand isolates agree reasonably well with de Hoog's

Plate XX

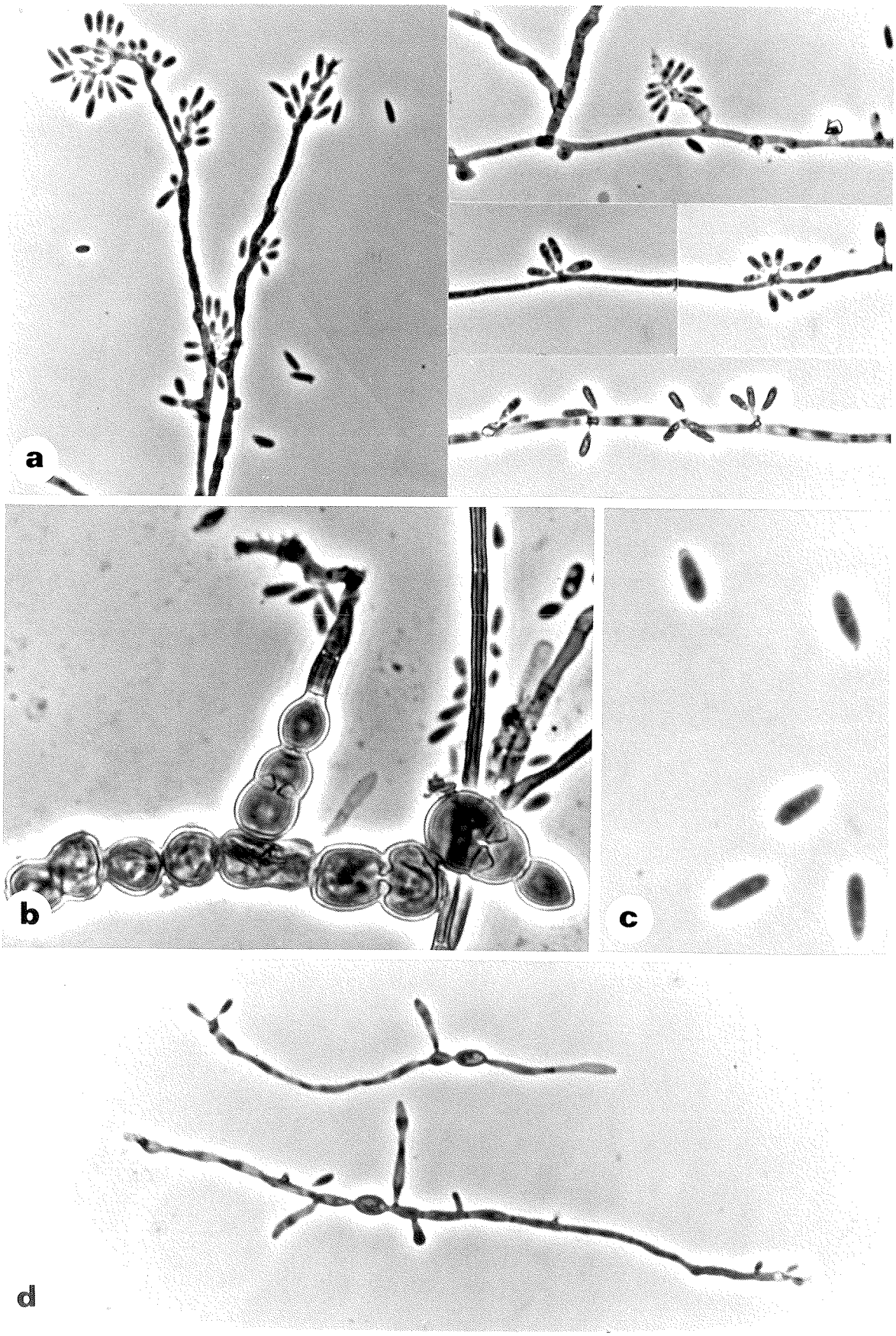
Leptodontidium elatius (Mangenot) de Hoog var. elatus de Hoog.

Fig. a. Conidiophores (X760).

Fig. b. Chlamydospores with conidiophore (X760).

Fig. c. Conidia (X1850).

Fig. d. Germinating conidia with conidiogenous cells (X760).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description of L. elatius var. elatius and the description given by Schol-Schwarz (1968), however a number of differences do exist.

Although Schol-Schwarz reported a phialidic state, phialides were not observed in our isolates and the growth rate was slower than that attributed to isolates belonging to this species by de Hoog and Schol-Schwarz. In addition, chlamydospores were found to be produced when the fungus was grown out onto glass slides for microscopic observation; neither de Hoog nor Schol-Schwarz report chlamydospores for L. elatius var. elatius but de Hoog does record chlamydospores being rarely produced in L. elatius (Mangenot) de Hoog var. ovalisporum de Hoog.

21. Leptographium sp. Plate XXI, Figs. a-d.

Colonies attaining a diameter of 50-60 mm in 12 days at 20°C on 2% malt agar. Colony appearing slimy and appressed except for the conidiophores; olive-brown at first, turning dark brown to black. Conidiophores mononematous, macronematous, erect; stipes dark-brown to black, 460-2,015 µm in length and 10-15 µm wide at the base. The sporogenous apparatus consists of a series of penicillate branches restricted to the apical region which terminate ultimately in the conidiogenous cells. Conidiogenous cells variable; (1) monoblastic, mostly discrete, arranged penicillately, percurrent, cylindrical or subulate; and (2) polyblastic, discrete, arranged penicillately on branches, sympodial, cylindrical or

subulate. Conidia chiefly produced as annellospores, more rarely as sympodulospores; hyaline; one-celled, oblong to slightly tapering towards the point of attachment and with obtuse ends; 2.8-5.2 x 1.2-2.4 µm.

NEW ZEALAND HOSTS: Larix sp., Pinus nigra Arnold and Pinus radiata D. Don.

CULTURES ISOLATED: R 5 isolated from Pinus radiata, Kaingoroa Forest; R 23 $(d)_I$ isolated from Larix sp., Waiotapu Forest; R 166 isolated from Pinus nigra, Kaingoroa Forest; R 174 $(a)^1$ isolated from Pinus nigra, Kaingoroa Forest.

For years, Leptographium spp. have been known to be important wood-staining fungi and many have their teleomorphs in the genus Ceratocystis.

Shaw and Hubert (1952) reviewed the taxonomy of the Leptographium complex, while Kendrick (1961, 1962, 1963, 1964) revised the taxonomy of this group, employing the type of conidiogenesis as the major criteria for separation of species. The result of these studies was that the number of true Leptographium species were reduced. The problem with separating species into different genera based on their type of conidiogenesis is that some species of this complex of fungi can exhibit more than one type of conidiogenesis on the same conidiophore. This was true of the New Zealand isolate which produced both annellospores and sympodulospores (Fig.c, Plate XXI), although the latter were much less abundant.

In researching the literature, comparisons were made between

Plate XXI

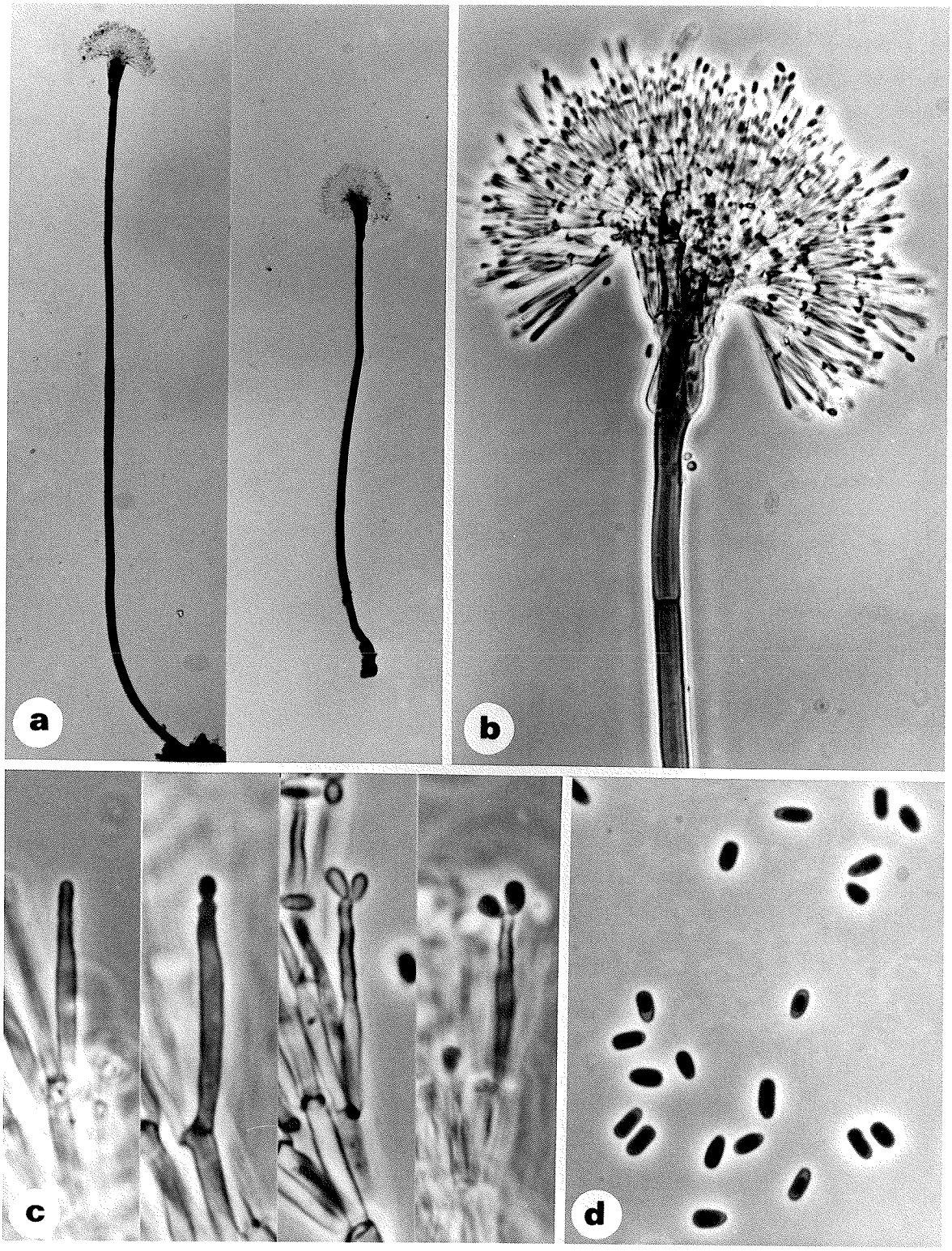
Leptographium sp.

Fig. a. Conidiophores (X105).

Fig. b. Apex of conidiophore with conidiogenous cells (X680).

Fig. c. Conidiogenous cells (X1900).

Fig. d. Conidia (X1650).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the isolates from New Zealand and Leptographium spp. described by other workers [Leptographium lundbergii Lagerb. & Melin in Lagerberg et al. (1927), Leptographium microsporum Davidson (1935), Leptographium engelmanni Davidson (1955), Leptographium terebrantis Barras & Perry (1971), Leptographium reconditum Jooste (1978)] in addition to the Leptographium anamorphs of Ceratocystis (see Upadhyay, 1981). While no claim is made that this is a complete listing, it appears that the New Zealand isolates differ from all of the above species by having an extremely long stipe in combination with conidia which are generally smaller than most but not all species of Leptographium. While the isolates from New Zealand could probably be described as a new species, for the present it will be listed simply as a Leptographium sp. until a more exhaustive literature search can be conducted.

Mammariopsis gen. nov.

Colonia floccosa atrocinerea vel nigra. Conidia trium formarum sequentium; arthroconidia holoblastica, semper intercalaria, pallido-brunnea, aut solitaria aut catenata; aleuriosporae monoblasticae polyblasticaeve, aut sessiles aut ex brevissimis conidiophoris exorientes, solitariae vel fasciculatae, plerumque unicellulares sed raro bicellulares, pallido-brunneae, rimam germinalem carentes; phialosporae hyalinae, unicellulares, ex conidiophoris similibus illis Phialemonii emissae.

TYPUS GENERIS: Mammariopsis variospora.

Colony floccose; dark grey to black. Three types of conidia produced. (1) Holoblastic arthroconidia which are solitary or produced in chains and always intercalary; light brown in colour. (2) Monoblastic to polyblastic aleuriospores which are sessile or arise from very short-stalked conidiophores; solitary or clustered; conidia one- to rarely two-celled; mid golden-brown in colour and lacking a germ slit. (3) Phialospores hyaline; one-celled and produced from a Phialemonium-like state.

TYPE SPECIES: Mammariopsis variospora.

22. Mammariopsis variospora sp. nov. Plate XXII, Figs. a-e.

Colonia in agaro cum extracto malti (2%) floccosa sed in medio adpressa; primo hyalina, postea in medio atromurina, demum post 12 dies nigra. Conidia trium formarum sequentium; arthroconidia holoblastica, semper intercalaria, pallido-brunnea, aut solitaria aut catenata, oblonga doliiforma, late complanata ubi ad cellules contiguas affixa, 4.0-10.0 x 3.0-5.5 µm; aleuriosporae monoblasticae polyblasticaeve, aut sessiles aut ex conidiophoris micronematis vel hemimacronematis exorientes, solitariae vel fasciculatae, raro in catenis brevibus lateralibus ex duobus vel tribus sporis constantibus, pallido-brunneae, nunc late ellipsoidales nunc ovales nunc naviculares nunc interdum fusiformes, semper late complanatae ubi in cellula conidiogena affixae, uni-

cellulares vel raro bicellulares, rimam germinalem carentes, 5.5-9.0 x 3.0-5.0 µm; phialoconidia enteroblastica aut ex phialidibus discretis aliquando ramosis cylindricus vel angustatis aut ex clavis cylindricis vel leviter angustatis emissa, hyalina unicellularia nunc ovalia nunc elliptica nunc allantoidea reniformiave nunc obpyriformia sed maxima pro parte oblonga, 2.0-4.5 x 0,75-2.0 µm.

TYPUS: 100 (e) sejunctus ex Pino radiata D. Don ex sylva Whangapouae. J. Reid legit May 19th, 1982.

Colonies attaining a diameter of 26-28 mm in 12 days at 20°C on 2% malt agar. Colony periphery floccose with an appressed centre; at first hyaline, the centre of the colony later turning very dark-grey then black. Hyphae variable; varying from thin-walled with normal appearing septa, hyaline to pale-brown in colour, 1.5-3.0 µm in diameter, to thick-walled, closely septate, dark-brown hyphae up to 5.0 µm in diameter with very thick septa up to 1.5 µm thick. Many of the hyphae appear to have what may be a mucilagenous sheath with varying amounts of material deposited thereon, giving a somewhat roughened appearance to the hyphae when they are observed at higher magnifications. The darker, broader hyphae sometimes adhere laterally, giving the appearance of rope-like structures.

Three types of conidia are produced: (1) Holoblastic arthroconidia which are solitary or produced in chains and always intercalary. They are oblong, doliiform, broadly flattened at their

points of attachment to adjacent cells; thick-walled; light brown in colour; 4.0-10.0 x 3.0-5.5 µm. (2) Monoblastic to polyblastic aleuriospores which are sessile or arise from very short-stalked conidiophores (micronematous to semi-macronematous); solitary or clustered, rarely in short-lateral chains of two or three conidia, conidia one to rarely two-celled; mid golden brown in colour and variable in shape; broadly ellipsoidal, oval, navicular or occasionally fusiform but always broadly flattened at the basal scar or point of attachment to the conidiogenous cell; thick-walled and smooth but, in age, sometimes appearing rugulose; germ slit absent; 5.5-9.0 x 3.0-5.0 µm. (3) A phialidic state also produced which forms conidia from two distinct types of conidiogenous cells: (a) discrete cylindrical to slightly tapering phialides; hyaline; usually lacking a collarette; sometimes sympodially branched, and up to 30 µm long, 0.5-1.0 µm wide at the tip and 1.0-2.0 µm wide at the base; usually separated from the subtending vegetative hyphal cell by a septum; or (b) hyaline, cylindrical to slightly tapered phialidic pegs; collarettes rare, and occasionally proliferating sympodially; 2.0-8.0 µm long and 1.0-2.0 µm wide at their base. The conidia are hyaline, one-celled and very variable in shape; oval, elliptical, allantoid to reniform, obpyriform, but chiefly oblong; 2.0-4.5 x 0.75-2.0 µm.
TYPE: 100 (e) isolated from Pinus radiata D. Don, Whangapoua Forest, New Zealand. Collected by J. Reid May 19th, 1982.
NEW ZEALAND HOST: Pinus radiata D. Don.

Plate XXII

Mammariopsis variospora sp. nov.

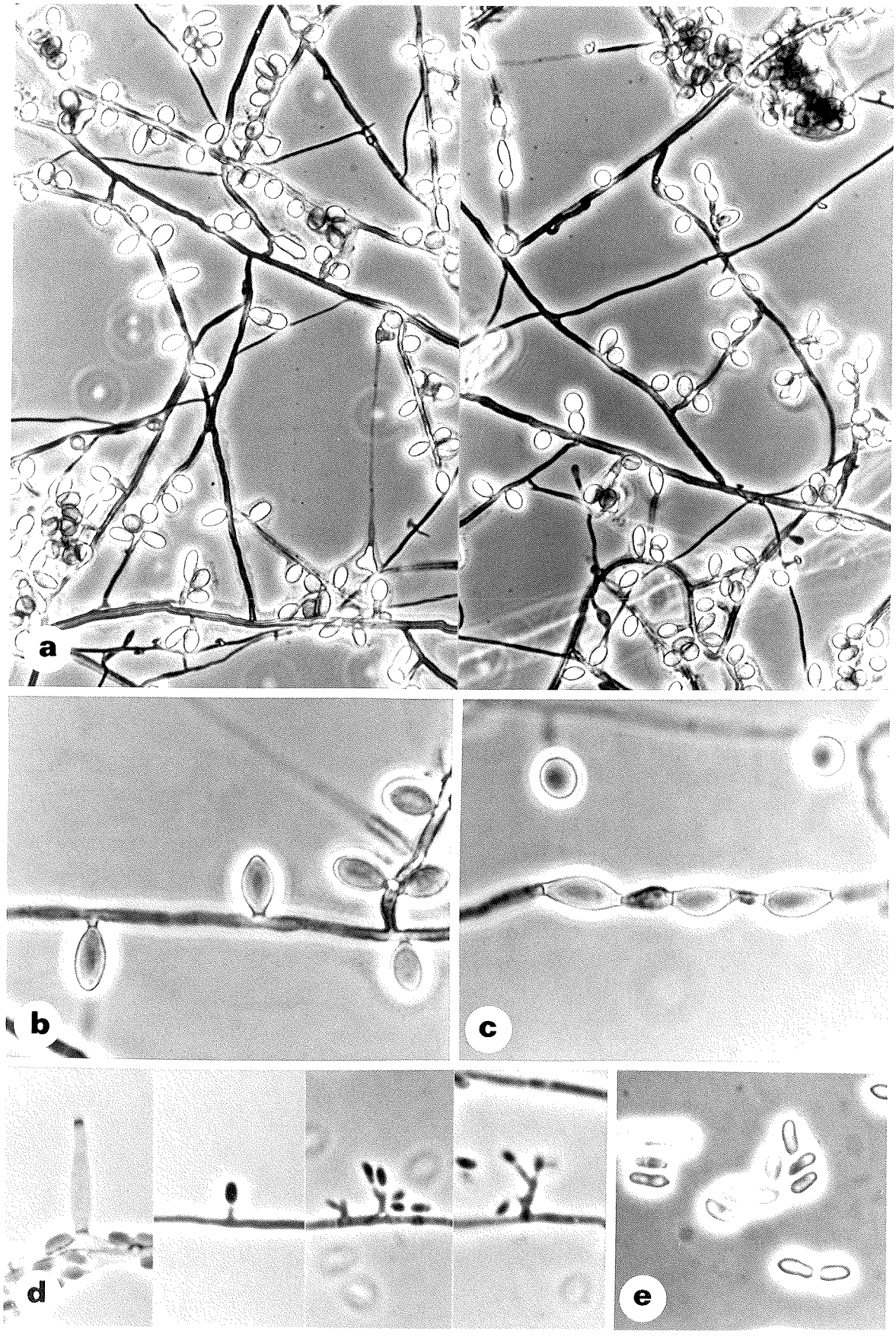
Fig. a. Mycelium with aleuriospores (X610).

Fig. b. Aleuriospores and conidiogenous cells (X1500).

Fig. c. Intercalary arthrospores (X1500).

Fig. d. Phialides; both simple, phialidic peg and branching phialides (X1500).

Fig. e. Phialospores (X1500).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CULTURES ISOLATED: 100 (a) isolated from Pinus radiata, Whangapoua Forest; 100 (e) isolated from Pinus radiata, Whangapoua Forest.

Two isolates of a wood staining hyphomycete were collected during this study which bore a superficial resemblance to the genus Mammaria Ces., a genus containing the single species Mammaria echinobotryoides Ces. However, it became apparent that not only was it different specifically from M. echinobotryoides but it possessed sufficient unique characteristics to warrant the erection of a new genus.

Not only does Mammariopsis variospora lack germ slits in the aleuriospores (found in M. echinobotryoides), but the aleuriospores are also smaller in size [5.5-9.0 x 3.0-5.0 µm vs. 8.5-17.5 x 4.5-8.5 µm] than those of M. echinobotryoides (Hennebert, 1968). Intercalary arthrospores are present in M. variospora while only terminal arthrospores occur in M. echinobotryoides. The phialidic states differ, as M. echinobotryoides possesses a Phialophora-like state (Hennebert, 1968) which produces globose conidia 1.5-1.8 µm in diameter while M. variospora possesses a Phialemonium-like state with variably shaped phialospores 2.0-4.5 x 0.75-2.0 µm. Phialospores of Mammariopsis variospora germinated very easily while Park (1973) found that phialospores of M. echinobotryoides failed to germinate on 13 types of agar media tested.

23. Oidiodendron rhodogenum Robak, Nyt. Mag. f. Naturw. 71: 254-255, 1932 Plate XXIII, Figs. a-c.

Colonies attaining a diameter of 8 mm in 12 days at 20°C on 2% malt agar. Colony flocculose, appressed at the edges; at first hyaline then turning grey to pinkish grey; a very prominent dull to bright red pigment diffusing into the agar around the colony. Conidiophores mononematous, macronematous; branched with a light coloured stipe; 45-260 µm long and 2.0-3.25 µm wide. Conidiogenous cells integrated, terminal on branches, determinate, fragmenting to form arthroconidia which develop in long branched chains, in which the individual spores are connected by peg-like structures. Conidia light-coloured and one-celled; subglobose, ellipsoidal to almost oblong; minutely verruculose; 2.0-3.1 x 1.25-2.0 µm.
NEW ZEALAND HOSTS: Larix sp., Pinus radiata D. Don.
CULTURES ISOLATED: 88 (a) isolated from Pinus radiata, Tairua Forest; 87 (cii) isolated from Pinus radiata, Tairua Forest; 158 (e) isolated from Larix sp., Waiotapu Forest; R 178 (a) isolated from Larix sp., Waiotapu Forest; R 180 (b) isolated from Larix sp., Waiotapu Forest.

This species of Oidiodendron Robak can be easily separated from the others in the genus by the deep red pigments which are produced. Robak (1932) reported that the ability of individual isolates to produce pigment varied from one generation to another. During the present study, successive generations of individual

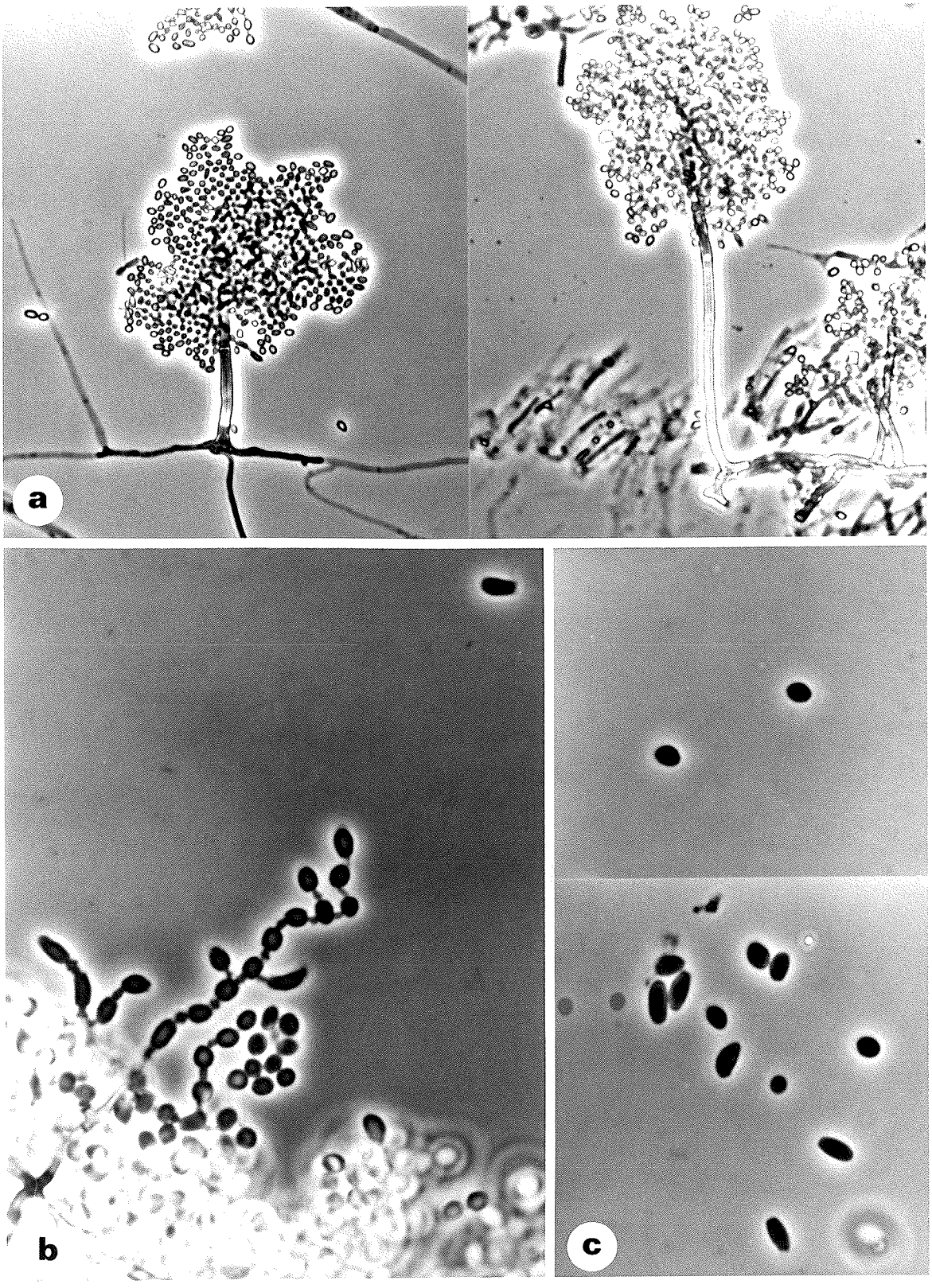
Plate XXIII

Oidiodendron rhodogenum (Robak)

Fig. a. Conidiophores (X680).

Fig. b. Chains of conidia with intercalary cells (X1650).

Fig. c. Conidia (X1650).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isolates did not vary in their pigment production, however, the intensity of the pigment produced varied from one isolate to another. The only difference found between the New Zealand isolates and those discussed by other researchers (Robak, 1932; Barron, 1962; Ellis, 1976) is the length of the conidiophores in the New Zealand isolates. They are up to 100 µm longer than previously reported.

24. Phialemonium dimorphosporum W. Gams & W.B. Cooke, apud Gams and McGinnis, Mycologia 75: 981, 1983 Plate XXIV, Figs. a-c.

Colonies attaining a diameter of 34-40 mm in 12 days at 20°C on 2% malt agar. Colony appressed to flocculose; at first hyaline, turning pinkish-grey then dark-grey. Conidiophores absent. Conidiogenous cells enteroblastic, phialidic, cylindrical in shape, with a discrete, often well developed collarette; phialides either peg-like or discrete and then Acremonium-like. Phialides hyaline, one-celled, and either allantoid 3.5-6.5 x 1.0-2.0 µm or ellipsoidal to oval 2.0-3.5 x 1.0-2.0 µm.

NEW ZEALAND HOSTS: Cupressus macrocarpa Hartw. and Pinus radiata D. Don.

CULTURES ISOLATED: 94 (a) isolated from Pinus radiata, Whangapoua Forest; R 100 (a) isolated from Pinus radiata, Whangapoua Forest; 100 (d) isolated from Pinus radiata, Whangapoua Forest; R 108 (b)

isolated from Cupressus macrocarpa, Woodhill Forest.

A number of isolates of what was believed to be a Phialophora sp. were isolated during this study. Although they lacked flask-shaped phialides, Cain (1952) pointed out that all types of gradations in the shape of the phialide had been found and the genus could not be sharply delimited in this respect. Cain also mentioned that in most species the phialide is usually separated from the parent cell by a septum, although such septa may be lacking, especially where the phialide is short (similar to the New Zealand isolates). With the publication of the paper by Gams and McGinnis (1983) it became apparent that these New Zealand isolates belonged to the new genus Phialemonium W. Gams & McGinnis as represented by the species P. dimorphosporum. As defined, Phialemonium possesses conidia which are usually found on lateral pegs of superficial or submerged hyphae and is intermediate in morphology between Acremonium and Phialophora Medlar. These phialidic pegs (called adelophialides) are not delimited from the subtending hyphal cell by a basal septum. Thus as Gams and McGinnis point out, the entire phialide is an intercalary hyphal cell with one or more lateral necks or pegs. These adelophialides are more commonly found than discrete longer phialides.

Although several minor differences exist between the New Zealand isolates and the species description in Gams and McGinnis for P. dimorphosporum, the New Zealand isolates are referred to this species. Well developed collarettes are sometimes found on

Plate XXIV

Phialemonium dimorphosporum W. Gams & W.B. Cooke

Fig. a. Conidiogenous cells (X1900).

Fig. b. Conidia with secondarily produced phialospores (X1900).

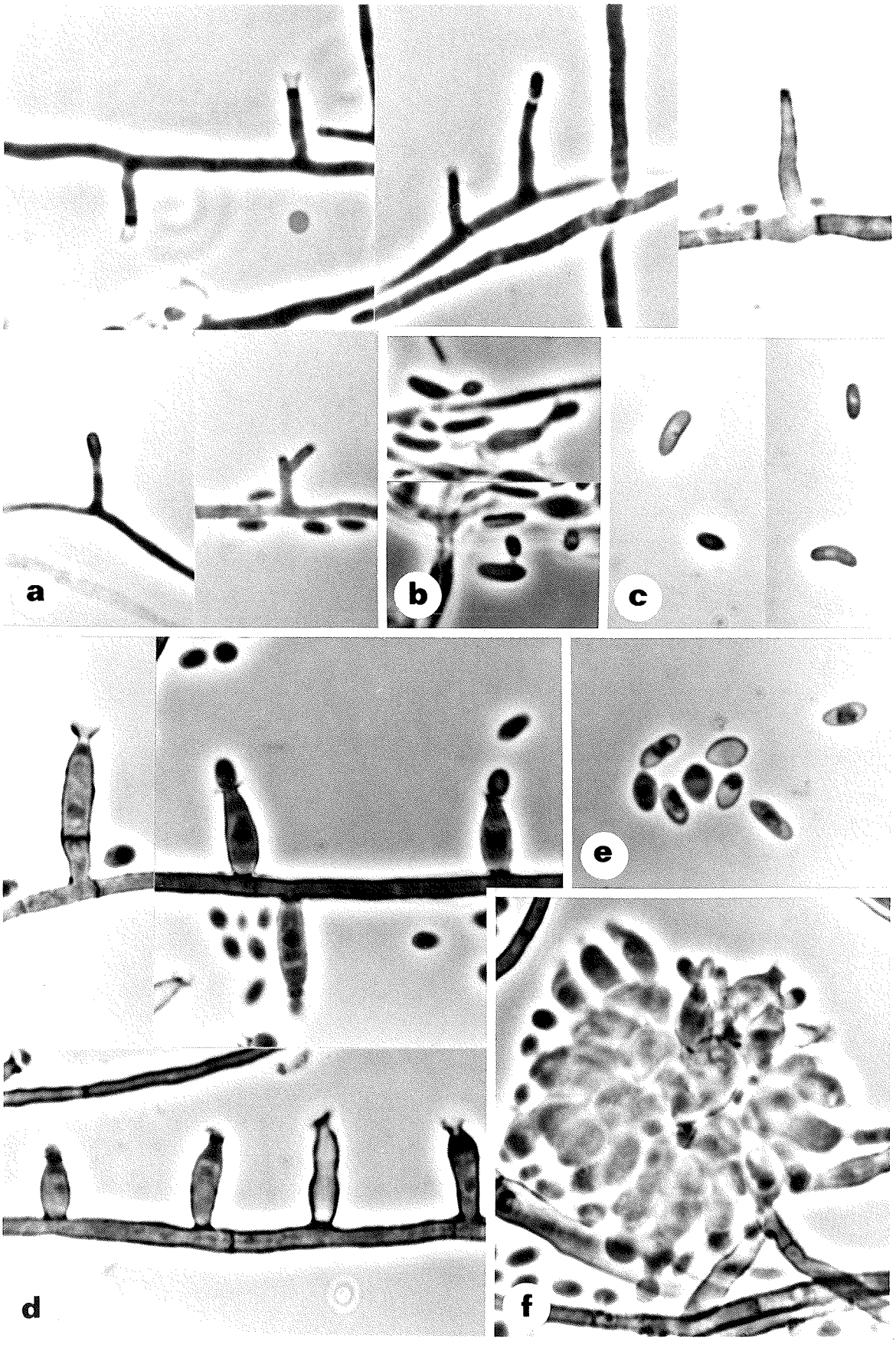
Fig. c. Dimorphic conidia (X1900).

Phialophora richardsiae (Nannf.) Conant

Fig. d. Phialides (X1750).

Fig. e. Conidia (X1750).

Fig. f. Clustered phialides (X1750).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the phialidic pegs of the isolates from New Zealand (Fig.a, Plate XXIV) contrary to the genus description given by Gams and McGinnis who noted that Phialemonium spp. lack conspicuous collarettes. Further, the phialidic pegs of P. dimorphosporum from New Zealand often show sympodial branching whereas the species as originally described lacks this. However it was found that the dimorphic conidia of the New Zealand isolate directly produced secondary conidia from small phialides typical of those noted by Gams and McGinnis who stated that repeated phialidic germination of conidia occurs in Phialemonium.

25. Phialophora richardsiae (Nannf.) Conant, Mycologia 29: 598, 1937 Plate XXIV, Figs. d-f.

≡ Cadophora richardsiae Nannf. apud Melin and Nannfeldt, Sv. Skogsvårdsf. Tidskr. 32: 421, 1934.

= Phialophora brunnescens (Davids.) Conant, Mycologia 29: 598, 1937 fide van Beyma (1943).

≡ Cadophora brunnescens Davids., J. Agric. Res. 50: 803, 1935.

= Phialophora caliciformis G. Smith, Trans. Br. mycol. Soc. 45: 391, 1962 fide Nicot and Caillat (1967).

Colonies attaining a diameter of 18-20 mm in 12 days at 20°C on 2% malt agar. Colony floccose; pale-brown turning to yellowish-brown. Conidiophores absent. Conidiogenous cells

enteroblastic, phialidic; hyaline, cylindrical to flask-shaped with a prominent, often flaring collarette; usually solitary and produced along hyphal elements, although sometimes produced in small to large groups, on a common stalk and then resembling small sporodochia. Phialides 6.0-20.0 µm long and 3.0-4.0 µm wide at widest point but 1.0-2.0 µm wide at tip. Conidia hyaline; one-celled, and either ellipsoidal to slightly allantoid 5.0-8.0 x 1.5-2.5 µm or subglobose 3.0-5.0 x 2.0-3.5 µm.

NEW ZEALAND HOSTS: Dacrydium cupressinum Lamb., and Podocarpus spicatus R. Br. ex Mirbel.

CULTURES ISOLATED: R 134 (e) isolated from Dacrydium cupressinum, Minginui Forest; R 147 (e) isolated from Podocarpus spicatus, Minginui Forest.

While the photographs of Kress et al. (1925), who worked in the U.S., prove they were the first to isolate Phialophora richardsiae, this fungus was first named and described by Nannfeldt (in Melin and Nannfeldt, 1934) based on a study of Swedish isolates. As Schol-Schwarz (1970) points out, the distinctive characteristics for this species are the sturdy phialides with broad, shallow cup-like collarettes with flaring margins and the presence of dimorphic conidia. Dimorphic conidia were first noted by Davidson (1935) in C. brunnescens [= P. richardsiae fide van Beyma (1943)] and since then have been used as a taxonomic character for this species by Nicot and Caillat (1967), Schol-Schwarz (1970) and Cole and Kendrick (1973). They all found that the hyaline,

allantoid to ellipsoidal conidia (primary phialoconidia of Cole and Kendrick) which were produced first, were followed in older cultures by subhyaline to light-brown conidia which were globose to subglobose in shape (secondary phialoconidia of Cole and Kendrick).

The observations of Cole and Kendrick indicated that the two kinds of conidia are associated with two distinct types of phialides which are produced at different stages of mycelial maturation. Primary phialides usually arise laterally from young vegetative hyphae and possess inconspicuous cupulate collarettes. Secondary phialides with flaring collarettes are usually produced singly and directly from maturing aerial hyphae. They noted that when the fungus is grown in slide culture, only primary phialides are formed. Although this study confirmed the observations of Cole and Kendrick, it was found that both primary and secondary conidia were produced when the New Zealand isolates were grown onto glass slides. Also the subglobose secondary conidia were hyaline rather than lightly pigmented as reported by most authors.

(e) Potential Staining Ability of Fungi Studied

As mentioned in the Methods and Materials, all members of the Ophiostomataceae isolated were included in this taxonomic

investigation regardless of their wood-staining ability. In addition, all other fungi associated with stained wood were isolated (other Pyrenomycetes, Coelomycetes and Hyphomycetes) and included, although no detailed attempt was made to describe the wood-staining ability of the various isolates. To do so would have required a very detailed undertaking similar to that reported by Lagerberg et al. (1927) and Goidanich (1936c). It would have required a large selection of different wood types for testing (hardwoods and softwoods both native and introduced to New Zealand) in a large number of replicates, and anatomical examination of the stained wood to observe critically the type of staining involved, e.g. diffusion of fungal pigments into the wood or penetration by dark coloured mycelium. The inoculations onto wood discs were mainly used as a technique for the induction of perithecia, staining of the wood being used simply as a criterion for including the organism in this study. This staining, no matter how little, is affected by the type of wood inoculated and the isolate itself. However, the following listing of the organisms studied which is based on the stain they imparted to the wood discs, does give some indication of the staining potential of the individual species.

Non staining Ophiostomataceae

Ceratocystiopsis falcata

Ceratocystis coronata

Ceratocystis piceae

Ceratocystis rostrocoronata

Sphaeronaemella fimicola

Brownish to blackish staining

Ceratocystis ips

Ceratocystis novae-zelandia

Ceratocystis piceaperda

Ceratocystis pilifera

Coniochaeta velutina

Cytospora sp.

Fusicoccum cf. tingens

Phoma sp.

Aureobasidium pullulans var. pullulans

Cladosporium cladosporioides

Cladosporium tenuissimum

Exophiala jeanselmei var. jeanselmei

Leptodontidium elatius var. elatius

Leptographium sp.

Mammariopsis variospora

Phialemonium dimorphosporum

Phialophora richardsiae

Red staining

Fusarium sp.

Oidiodendron rhodogenum

Yellow to brownish staining

Hyalopesotum pini

(B) INVESTIGATION INTO PERITHECIAL INDUCTION OF CERATOCYSTIOPSIS FALCATA

METHODS AND MATERIALS

Under natural conditions, our isolates of the teleomorph of C. falcata only occurred in association with other fungi, particularly Gliocladium roseum. To investigate what the stimulating principle might be, culture extracts were obtained from both shake and still cultures of G. roseum, and these incorporated into a medium onto which C. falcata was then inoculated according to the protocol detailed in Figure 7.

Conidia of the G. roseum were inoculated into acid washed flasks containing modified Robinson's medium (McMillan, 1980). After the appropriate incubation periods, the cultures were filtered through 0.45 µm Millipore filters in Falcon sterile, disposable 150 ml filter units (Becton, Dickinson and Co., Oxnard, California), and the sterile filtrates were retained. These filtrates, as well as the vitamins in two concentrations and in various combinations as shown in Table 2, were incorporated into separate flasks of molten agar containing modified Robinson's medium and mixed thoroughly; final concentration being 20 g/l of agar. These various molten agars were now poured into separate petri plates, each treatment being replicated 8 times or more. They were then inoculated with C. falcata.

Later observations of the plates indicated that the filtrates from the 8-day old G. roseum cultures (no significant difference between shake and unshaken cultures), stimulated the most perithecial development while the vitamins had no effect. Therefore a supply

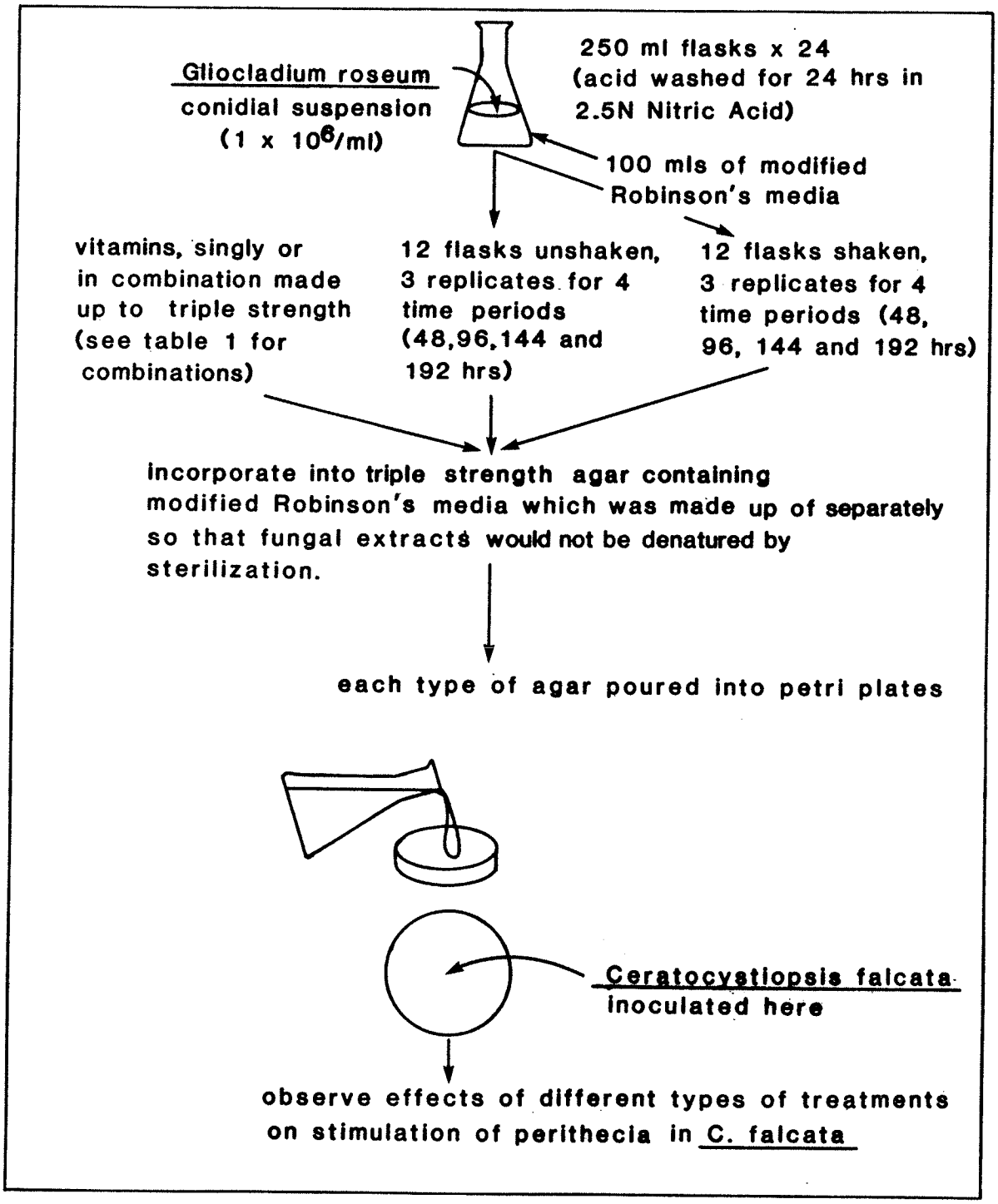

Figure 7. Outline of treatments used to determine the nature of Gliocladium roseum extracts stimulating perithecial induction in Ceratocystiopsis falcata

Table 2. Treatments to induce formation of perithecia in *Ceratocystiopsis falcata*

Treatment incorporated into modified Robinson's medium agar
filtrate from (48 hrs) shaken culture
" " (96 hrs) " "
" " (144 hrs) " "
" " (192 hrs) " "
" " (48 hrs) unshaken culture
" " (96 hrs) " "
" " (144 hrs) " "
" " (192 hrs) " "
control (uninoculated)
thiamine (100 µg/l)
pyridoxine (100 µg/l)
biotin (5 µg/l)
thiamine, pyridoxine
thiamine, biotin
pyridoxine, biotin
thiamine, pyridoxine, biotin
control (no vitamins)
thiamine (10 mg/l)
pyridoxine (10 mg/l)
biotin (0.5 mg/l)
thiamine, pyridoxine
thiamine, biotin
pyridoxine, biotin
thiamine, pyridoxine, biotin
control (no vitamins)
filtrate (192 hrs) heated at 100°C
" " " at 65°C
" " not heated

of filtrate from 8-day old shake cultures was obtained and frozen until ready to be analyzed. Samples of 192 hour filtrate were heated at 65^{o}C and 100^{o}C for 30 minutes, and incorporated into agar media as explained above. When these plates were poured and hardened, they were inoculated with C. falcata in order to observe whether the filtrate was still active.

RESULTS AND DISCUSSION

When grown *in vitro*, New Zealand isolates of *Ceratocystiopsis falcata* failed to produce any perithecia, even after one year. However, when grown in association with other fungi, particularly *Gliocladium roseum* which was always associated with *C. falcata* in our original collections, perithecial production was initiated. Similar observations were made by Rayner and Hudson (1977) with respect to British isolates of this fungus, which were stimulated to produce perithecia *in vitro* when grown in mixed culture with *Acremonium butyri* (van Beyma) Gams or *Trichoderma* spp. Their paper indicated that close mycelial contact between the fungi was necessary since when culture extracts of *A. butyri* and *Trichoderma* spp. were added to media on which *C. falcata* was growing, they failed to stimulate production of perithecia. While no details were given as to their procedures, Hudson (pers.comm.) stated that culture extracts of *A. butyri* and *Trichoderma* spp. grown in 2% malt extract for 7 days at 20^{o}C were millipore filtered, then the filtrate was added to molten (50^{o}C) 2% malt extract agar immediately before pouring and inoculation with *C. falcata*.

As outlined earlier, a similar procedure was carried out during this study with the result that filtrates from 8-day-old liquid cultures of *G. roseum*, incorporated into the agar medium, initiated perithecial production in *C. falcata*. Because a different test organism was used from that of Rayner and Hudson, an analogy can not be made. However, this present study does clarify two points:

(1) mycelial contact between the fungi is not needed for perithecial initiation and; (2) there is a substance or substances being leached by the G. roseum which initiates perithecial formation in C. falcata when present in higher concentrations (filtrates from cultures incubated for longer time periods having the greatest effect).

Because earlier studies with species of the related genus Ceratocystis showed B vitamins were necessary for perithecial induction (Robbins and Ma, 1942a, 1942b and 1943; Käärik, 1960), it was thought that C. falcata possibly possessed such a vitamin deficiency. Thus the three vitamins biotine, pyridoxine and thiamine, either singly or in various combinations and in two concentrations (see Table 2) were added to agar media onto which C. falcata was later inoculated. As perithecial production was not induced, even after one year, it seemed that the stimulating substance(s) in the filtrate of G. roseum must be something other than a B vitamin. None of the species of Ceratocystis recently transferred to Ceratocystiopsis by Upadhyay (1981) were among those investigated by earlier workers in regard to their vitamin requirements. However, if Ceratocystiopsis does in fact represent a distinct group of organisms, then perhaps physiological differences in addition to morphological differences exist between the species of the two genera.

Since a B vitamin deficiency was not apparent in C. falcata, it could be something else that acted as a stimulating substance or substances in the G. roseum filtrate. Results showed that when the filtrate was heated at temperatures of 65^{o}C and 100^{o}C, the stimulatory

substance(s) was inactivated as no perithecia were produced by C. falcata isolates when grown on media containing filtrates heated to these temperatures. Proteins are substances which are easily denatured by heat, and although these results are certainly not conclusive, it is quite possible the stimulatory substance(s) is a simple protein of low molecular weight; low molecular weight because this protein(?) must be small enough to enter through the cell walls of C. falcata before initiating the appropriate biochemical process leading to perithecial production.

Perhaps this process can be explained by the operon theory which is an explanation of the genetic control of developmental processes found in cells. As outlined by Bidwell (1979) it states that structural genes which programme mRNA for specific enzymes, occur singly or in groups in combination with an operator gene. This operator gene functions to maintain the structural gene either in the active or open state or in the inactive or closed state. The combination of structural gene and operator gene in cells is called an operon. A separate regulator gene (not part of the operon) forms a regulating molecule called the repressor that maintains the operator gene in the closed state, thus holding the operon inactive. The presence or addition of a molecule called the inducer, which combines with or inactivates the repressor, allows the operator gene to go into the open state, thus activating the operon. Bidwell (1979) believes that these inducer molecules may be simple metabolites involved in specific reactions or metabolic sequences which result in an orderly process of growth and differentiation. Thus it is

possible that the stimulating substance in the extract of G. roseum acts as an inducer to inactivate a repressor gene in C. falcata and allow the operator gene to activate the operon. The operon would thus produce enzymes which would induce the production of perithecia. The operon theory, though presently unproven, could explain the results of these experiments.

Observations have shown that C. falcata responds to the extracts of G. roseum in other ways besides the production of perithecia. In pure culture the Chalara state agrees well to that described by Rayner and Hudson (1977) but when grown on agar medium with G. roseum extract incorporated into it, a second Chalara state is formed which is associated only with the perithecia. It is not generally spread over the colony as is the smaller Chalara state. It is not that these phialides are longer, as the measurements for both Chalara states overlap, but they are wider and produce conidia which are far larger in size relative to those produced by the normal sized phialides. Wherever perithecia form, the adjacent mycelium takes on a darker colour as do the phialides of the associated larger Chalara state.

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APPENDICES

(A) Key to the Species of Ophiostomataceae Isolated During this Study

1. Ascocarps pale yellow to orange in colour.....
.....*Sphaeronaemella fimicola*

   Ascocarps dark brown to black in colour.....2

2. Ascospores falcate in side view.....*Ceratocystiopsis falcata*

   Ascospores not falcate in side view.....3

3. Ascospores with sheath appearing cucullate in side view, quadrangular in plan view and triradiate in end view
.....*Ceratocystis piceaperda*

   Ascospores not as above.....4

4. Ascospores with sheath appearing in side and plan view slightly concave in the long axis of the spore, convex in the short axis, but projecting in the corners, quadrangular in end view but with slightly concave surfaces.....*Ceratocystis ips*

   Ascospores not possessing a sheath.....5

5. Perithecial necks up to 10 mm or more in length *in vitro*
.....*Ceratocystis novae-zelandiae*

   Perithecial necks not exceeding 3 mm *in vitro*.....6

6. Possesses both *Pesotum* and *Sporothrix*-like to *Hyalodendron*-like anamorph.....*Ceratocystis piceae*

   Possesses only *Sporothrix*-like to *Hyalodendron*-like anamorph.....7

7. Colonies on 2% malt agar white in colour...*Ceratocystis coronata*

   Colonies on 2% malt agar pigmented.....8

8. Perithecia developing slowly and sparsely in fresh isolates on 2% malt agar, developing quickly and abundantly on sterilized wood.....*Ceratocystis rostrocoronata*

   Perithecia developing quickly and abundantly in fresh isolates on 2% malt agar (provided both mating types present).....*Ceratocystis pilifera*

(B) Scientific and Common Names of Tree Hosts mentioned in the Text

Beilschmiedia tawa	- Tawa
Cupressus macrocarpa	- Monterey Cypress
Dacrydium cupressinum	- Rimu, Red Pine
Eucalyptus sp.	- Gum tree
Larix sp.	- Larch, Tamarack
Pinus elliotii	- Slash Pine
Pinus nigra	- Corsican Pine
Pinus radiata	- Radiata Pine
Pinus taeda	- Loblolly Pine
Podocarpus spicatus	- Matai, Black Pine
Pseudotsuga menziesii	- Douglas Fir

(C) Other Fungi Isolated During Study

1. Acremonium spp.

   NEW ZEALAND HOSTS: Cupressus macrocarpa, Eucalyptus sp., Pinus elliotii, Pinus radiata, Podocarpus sp.

2. Arthrobotrys oligospora Fres., Beitr. Mykol. 1: 18, 1852.

   NEW ZEALAND HOST: Pinus radiata.

3. Beauveria sp.

   NEW ZEALAND HOST: Pinus nigra

4. Chalara sp.

   NEW ZEALAND HOST: Podocarpus sp.

5. Cylindrocarpon destructans (Zins.) Scholten, Neth. J. Plant Path., 70, suppl. 2: 9, 1964.

   NEW ZEALAND HOSTS: Beilschmiedia tawa, Dacrydium cupressinum, Podocarpus spicatus.

6. Cylindrocarpon didymum (Hartig) Wollenw., Fus. autogr. del., ed. 2, 650, 1926.

   NEW ZEALAND HOSTS: Pinus radiata, Podocarpus sp.

7. Cylindrocarpon gracile Bugn., Encycl. mycol., 11: 162-164, 1939.

   NEW ZEALAND HOST: Eucalyptus sp.

8. Cylindrocarpon spp.

   NEW ZEALAND HOSTS: Pinus radiata, Podocarpus spicatus.

9. Cylindrocladium parvum Anderson, Mass. Agric. Exp. Sta. Bull. 183: 37, 1919.

NEW ZEALAND HOST: Pinus radiata.

10. Cyphellophora(?) sp.

NEW ZEALAND HOST: Dacrydium cupressinum.

11. Fusarium culmorum (W.G. Smith) Sacc., Syll. Fung. 2: 651, 1885.

NEW ZEALAND HOST: Larix sp.

12. Fusarium solani (Mart.) Sacc., Michelia 2: 296, 1881.

NEW ZEALAND HOSTS: Cupressus macrocarpa, Pinus elliotii, Pinus radiata.

13. Fusarium spp.

NEW ZEALAND HOSTS: Beilschmiedia tawa, Cupressus macrocarpa, Eucalyptus sp., Pinus radiata.

14. Geotrichum candidum Link ex Pers. Mycol. eur. 1: 26, 1822.

NEW ZEALAND HOSTS: Pinus nigra, Pinus radiata.

15. Geotrichum sp. nov. (?)

NEW ZEALAND HOST: Pinus radiata.

16. Geotrichum sp.

NEW ZEALAND HOST; Pinus radiata.

17. Gliocladium roseum (Link) Bainier, Bul. Soc. Mycol. France 23: 111-112,1907.

NEW ZEALAND HOST: Larix sp.

18. Gliocladium spp.

NEW ZEALAND HOSTS: Cupressus macrocarpa, Eucalyptus sp., Larix sp., Pinus elliotii, Pinus radiata, Podocarpus spicatus.

19. Hyalodendron lignicola Diddens, Zentralb. Bakt. Parasit. Infektionskr. Abt. II, 90: 317-318, 1934.

NEW ZEALAND HOST: Pinus radiata.

20. Hyalorhinocladiella sp.

NEW ZEALAND HOST: Pinus radiata.

21. Lecythophora sp.

NEW ZEALAND HOST: Pinus radiata.

22. Oedocephalum(?) sp.

NEW ZEALAND HOST: Eucalyptus sp.

23. Oidiodendron tenuissimum (Peck) Hughes, Can. J. Bot., 36: 790, 1958.

NEW ZEALAND HOSTS: Pinus elliotii, Pinus radiata.

24. Penicillium sp.

NEW ZEALAND HOST: Larix sp.

25. Pesotum sp. (bicoloured synnemata)

NEW ZEALAND HOST: Eucalyptus sp.

26. Pestalotiopsis sp.

NEW ZEALAND HOST: Cupressus macrocarpa, Pinus radiata.

27. Phialophora bubakii (Laxa) Schol-Schwarz, Persoonia, 6: 66, 1970.

NEW ZEALAND HOST: Pinus radiata.

28. Phialophora spp.

NEW ZEALAND HOSTS: Pinus radiata, Podocarpus sp., Pseudotsuga menziesii.

29. Seiridium unicorne (Cke & Ell.) Sutton, Mycol. Pap. 138: 74, 1975.

NEW ZEALAND HOST: Cupressus macrocarpa.

30. Sphaeridium(?) sp.

NEW ZEALAND HOST: Podocarpus spicatus.

31. Stilbum sp.

NEW ZEALAND HOST: Larix sp.

32. Trichoderma sp.

NEW ZEALAND HOST: Pinus radiata.

33. Verticillium spp.

NEW ZEALAND HOSTS: Beilschmiedia tawa, Larix sp., Pinus elliotii, Pinus radiata, Pseudotsuga menziesii.